SANDOZ

US FOOD & DRUG ADMINISTRATION

FDA Oncologic Drugs Advisory Committee Meeting

ZARXIO® (filgrastim)

January 7, 2015

ADVISORY COMMITTEE BRIEFING MATERIALS: AVAILABLE FOR PUBLIC RELEASE

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List of Abbreviations

ACA	Affordable Care Act
AE	Adverse event
ANC	Absolute neutrophil count
API	Active pharmaceutical ingredient
AUC	Area under the curve
AUEC	Area under the effect curve
b.w.	Body weight
BLA	Biologics License Application
BPCI	Biologics Price Competition and Innovation Act
CD	Circular dichroism
CEX	Cation exchange chromatography
CI	Confidence interval
C_{max}	Maximum serum concentration
CPA	Cyclophosphamide
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
DSN	Duration of severe neutropenia
ECOG	Eastern Cooperative Oncology Group
EMA	European Medicines Agency
E_{max}	Maximum ANC
EU	European Union
FAS	Full analysis set
FDA	US Food and Drug Administration
FN	Febrile neutropenia
G-CSF	Granulocyte colony-stimulating factor
G-CSFR	Granulocyte colony-stimulating factor receptor
GLP	Good laboratory practice
HSQC	Heteronuclear Single Quantum Coherence
i.v.	Intravenous
IeF	Isoelectric focusing

IND	Investigational new drug
K_D	Dissociation constant
MIU	Million units
MS	Mass spectrometry
NAB	Neutralization antibody assay
NMR	Nuclear magnetic resonance
ODAC	Oncologic Drugs Advisory Committee
PASS	Post-authorization safety study
PBPC	Peripheral blood progenitor cells
PD	Pharmacodynamics
PFS	Pre-filled syringe
PK	Pharmacokinetics
PP	Per-protocol
PPM	Parts per million
PSUR	Periodic safety update reports
rhG-CSF	Recombinant human granulocyte colony-stimulating factor
RIP	Radioimmunoprecipitation
RP-HPLC	Reversed-phase high-performance chromatography
s.c.	Subcutaneous
SAE	Serious adverse event
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEC	Size exclusion chromatography
SPR	Surface plasma resonance
TAC	Taxolene (docetaxel), Adriamycin (doxorubicin), and cyclophosphamide
TK	Toxicokinetics
US	United States
UV	Ultraviolet

1 Executive summary

The Sandoz group of companies ("Sandoz") is the pioneer in the development and marketing of biosimilar drugs and is honored to be the sponsor of the first biosimilar drug to be reviewed by an FDA Drugs Advisory Committee. Because of the novelty of this submission and the paradigm shift in thinking required for its review, this briefing book provides data and analyses, as well as perspective on how they align with the novel US regulatory requirements for biosimilar products. Of note, ZARXIO® has been marketed (under the tradename ZARZIO®) in Europe since 2009 as well as in several other highly regulated countries. The market experience with ZARXIO outside of the US, includes in excess of 7.5 million days of patient exposure; demonstrating its clinical safety and efficacy.

Filgrastim is a granulocyte-colony stimulating factor (G-CSF) produced by recombinant DNA technology. It has a well characterized structure and established mechanism of action; making it an appropriate molecule for the first biosimilar review. In order to establish biosimilarity and to support the licensing of ZARXIO (Sandoz biosimilar filgrastim), Sandoz has conducted an in-depth development program consistent with FDA guidance to demonstrate similarity in the structure and function of the product with its reference drug, Neupogen®.

The ZARXIO development program aligns with the stepwise approach outlined by the draft FDA guidance documents for establishing biosimilarity. Fundamental to meeting the statutory requirements for biosimilarity, the proposed product must be shown to be sufficiently similar to the reference drug to be confident that it will have no clinically meaningful differences. To this end, the structure and function of ZARXIO have been characterized using a number of highly sensitive orthogonal analytical methods and shown to be highly similar to the reference product.

Once similarity was confirmed using robust analytical methods, a focused pre-clinical and clinical program was used to provide the final confirmation of the similarity of the product to the reference drug. The totality of the data package including analytical, functional, preclinical and clinical information allows for a determination of biosimilarity.

The ZARXIO clinical trial program for the US application focuses on two pivotal studies. One PK/PD study (Study 109, study code: EP06-109) established equivalence and one clinical safety and efficacy study (Study 302, study code EP06-302) demonstrated the same clinical performance as the reference product. Both studies demonstrated equivalent pharmacodynamic performance. The breast cancer safety and efficacy study comparing ZARXIO to Neupogen included multiple switches of the two products (ZARXIO to Neupogen and back) to ensure there were no treatment emergent issues or clinically relevant immunogenicity precipitated by switching. The clinical safety and efficacy study also included a sub-set of patients evaluating pharmacokinetics. The clinical package is also supported by a global program including five randomized, double-blind, single and multiple dose PK/PD studies in healthy volunteers to assess pharmacokinetic and pharmacodynamic equivalence between ZARXIO and Neupogen and a European non-comparative clinical safety and efficacy study. As this product has been on the market since 2009, there are also ongoing pharmacovigilance activities and a non-comparative post-authorization safety study, all of which provided confirmation of similarity in clinical performance.

Biosimilars offer an important benefit to the public; their availability in the US will lead to increased patient access to biologic medicines and significant savings for healthcare systems that enable the sustainability of medical innovation as has already happened in Europe.

2 Introduction

On January 7, 2015 the Oncologic Drugs Advisory Committee (ODAC) is to provide their recommendations to the FDA on the approvability of ZARXIO (filgrastim), the first biosimilar biologic license application (BLA) to be reviewed by the US FDA.

Sandoz (a division of Novartis) is a pioneer in the biosimilar field, having started its biosimilars program in 1996, based on extensive in-house experience manufacturing innovative biologics. Sandoz launched the first-ever biosimilar product (recombinant human growth hormone, somatropin) in countries of the EU in 2006, followed by recombinant human erythropoietin (epoetin alfa) in 2007 and filgrastim in 2009. Based on this expertise and insight, Sandoz is well positioned to participate in this evaluation of the first biological drug to be reviewed in the US via the biosimilar pathway.

While there are presently no biosimilars approved in the US, the framework for such an approval is not novel. In fact, the standards recently established by FDA to ensure the safety and effectiveness of biosimilars are rooted in the long standing expertise the Agency has used when evaluating the comparability of the quality, safety and efficacy data following changes in manufacturing processes.

As the biosimilarity paradigm is new, this document not only provides an overview of the ZARXIO development program, but also a background on the evolution of the biosimilar concept and how the ZARXIO studies align with these standards to demonstrate biosimilarity to Neupogen.

2.1 Regulatory framework for the approval of biosimilar drugs

Until the passage of the Affordable Care Act (ACA) in March 2010, there was no provision in the US enabling FDA licensure of biological products based on comparison to an already licensed biological product, similar in concept to how generic drugs are approved for small molecule drugs. The *Biologics Price Competition and Innovation Act of 2009* (BPCI Act) was approved with the ACA in 2010 and provides a framework for a licensure pathway for biological products that are "biosimilar" to already licensed biological products that are currently on the US market. Under this law, a biological product may be demonstrated to be "biosimilar" if data show that, among other things, the product is "highly similar" to an already-approved biological product notwithstanding minor differences in clinically inactive components, and for which there is an expectation that there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product. Congress deferred to the FDA to develop and implement regulations and guidance documents for demonstrating biosimilarity. The agency has issued numerous draft guidance documents for industry, which provide a roadmap for the development and approval of biosimilar drugs.

For reference, there have long been provisions in the Food Drug and Cosmetic Act to provide a pathway for traditional small molecule drugs that are proven to be "the same" as an innovator drug. These are known as generic drugs, and by definition, must have "the same" active ingredient(s), previously approved conditions of use, dosage form, strength, route of administration, and (with certain exceptions) labeling as the reference listed drug. These "copies" get to market by relying on the FDA's previous findings that the reference listed drug, already approved under the Act, is safe and effective. The generic drug must also be clinically bioequivalent to the brand name drug.

It is generally straight forward to establish "sameness" for traditional small molecule synthetic drugs as one can synthesize an "identical" molecule to the reference compound. However, there are examples of more complex generic drugs, like enoxaparin, that were approved through the generic drug pathway that establish learnings applicable to biosimilars. In the enoxaparin case, FDA developed a scientifically rigorous approach based on multiple criteria to make a determination of "sameness" (Lee 2013). Enoxaparin is a low molecular weight heparin derived from porcine intestines. It is a complex mixture of molecules of various lengths and structures - it is not a single entity FDA used multiple criteria including analytical and functional data to determine that the distribution of the proposed generic enoxaparin was the "same" as the reference product. "Sameness" in a regulatory sense is not "identical." Generic enoxaparins have been approved using this scientific approach without FDA requiring any comparative clinical trials. With approval of generic enoxaparin, all indications of the reference product were granted based on the thoroughness of the data demonstrating molecular "sameness" and bioequivalence.

Other learnings that apply to the biosimilar concept include experience with manufacturing changes for originator biologic drugs. While the concept of similarity appears unique, it is important to note that almost identical logic applies to how manufacturing changes over time are dealt with for biological products. While well known to health authorities and manufacturers, it is not readily apparent to health care professionals and the public that product attributes of biologics may vary significantly over their manufacturing lifetime, due to the intrinsic variability of different batches of product (that must fit predetermined release specifications) as well as the larger variations that occur with manufacturing changes (which require approval by regulatory authorities) such as changes in cell lines, manufacturing sites and processes) (Scheistl 2011). In many cases, the resulting differences in individual product attributes can be as large, or larger than, the differences between a proposed biosimilar product and its reference. Fortunately, regulatory science has dealt with changes of this type for many years and has embraced modern analytics as a sufficiently sensitive tool to evaluate and determine whether the post-manufacturing change product is the "same" as the pre-manufacturing change product. Sometimes analytical data identify product attributes are sufficiently different that there is residual uncertainty whether the new products will perform similarly in the same in the clinical setting. In these cases, regulatory authorities ask for non-clinical and/or clinical data to confirm that the product attribute differences are not clinically relevant.

Therefore, the FDA has prior experience with the determination of "sameness" through the review and approval of complex generics and major manufacturing changes for originator biologics. Although the BPCIA regulatory pathway for review and approval of biosimilars was established only in 2010, these prior experiences can be applied to the review of biosimilars.

This being said, to most clinicians, biosimilar development and approval requires a paradigm shift in understanding, particularly when compared directly to novel drug development. The bulk of evidence for approval for a novel drug is centered on the clinical trial program providing robust evidence of safety and efficacy. In contrast, the goal of a biosimilars development program is to demonstrate high similarity to an approved reference product that has an established safety and efficacy profile. The bulk of evidence for approval of a biosimilar depends on a robust foundation of analytical and functional characterization data demonstrating that the drug substance is "essentially the same" active ingredient as the reference drug. The clinical trial program establishes pharmacokinetic bioequivalence and confirms similarity, but may appear insufficient compared to the originator's clinical trial program which had a completely different purpose. This is particularly the case when a biosimilar sponsor seeks extrapolation to all indications of the reference product. Clinicians expect clinical data in every indication for which a drug is approved. Yet, for a biosimilar, approval in all indications is based on the totality of the data demonstrating "sameness" to the reference product which has already proven safety and efficacy in all approved indications. Therefore, this briefing book by necessity provides copious information about the development and analytical characteristics of the molecule demonstrating "high similarity" to the reference product, Neupogen.

Finally, the BPCI Act stipulates that products submitted as biosimilars can be approved by the FDA as biosimilar or interchangeable biosimilars. At this time there is no guidance from the FDA distinguishing the two but biosimilar biological products cannot be dispensed in place of the originator biological product unless a physician or other healthcare professional prescribes the biosimilar product. Biological products will have to be shown to be "interchangeable" before they can be automatically substituted at the pharmacy. It is important to note that FDA desires a two-step process – first approval of biosimilarity followed by a subsequent submission for interchangeability. **The current BLA for ZARXIO seeks only approval of biosimilarity and is not seeking approval of interchangeability at this time**.

2.2 ZARXIO (filgrastim) overview

ZARXIO (filgrastim) is a proposed biosimilar of Amgen's Neupogen® (BLA 103353; 20 Feb 1991) and, based on its demonstrated similarity, Sandoz is seeking the approval of all five indications included in the labeling for Neupogen:

- Cancer patients receiving myelosuppressive chemotherapy
- Patients with acute myeloid leukemia receiving induction or consolidation chemotherapy
- Cancer patients receiving bone marrow transplant
- Patients undergoing peripheral blood progenitor cell collection and therapy
- Patients with severe chronic neutropenia

Filgrastim is produced by recombinant DNA technology (rhG-CSF). Compared to many other biological products, most notably monoclonal antibodies, filgrastim is a relatively simple, non-glycosylated protein. Using state-of-the-art analytical methods, the structure and function of ZARXIO have been confirmed with a high degree of confidence to be biosimilar to the reference product, US-licensed Neupogen. In addition, the robust development program establishing biosimilarity includes: five animal studies to assess pharmacodynamics, toxicity, toxicokinetics, and local tolerance; one pivotal and four supportive studies comparatively assessing the pharmacokinetics and pharmacodynamic effects of ZARXIO and Neupogen; and a pivotal clinical trial in breast cancer patients in which non-inferiority in clinical effectiveness was demonstrated. In all of these analyses a high degree of similarity, consistent with the regulatory standard, was demonstrated. Furthermore, supportive data were derived from the European application, which included a non-comparative clinical trial and non-comparative post-market study, and from post-marketing pharmacovigilance surveillance.

G-CSF belongs to the large family of hormone-like growth factors which are required for the growth and development of hematopoietic cells. G-CSF levels moderate granulopoiesis and and neutrophil maturation in response to stress conditions, such as bacterial infection. Use of G-CSF in patients undergoing cytotoxic chemotherapy leads to significant reductions in the incidence, severity and duration of neutropenia and febrile neutropenia. Use of G-CSF, either alone or after chemotherapy, mobilizes hematopoietic progenitor cells into the peripheral blood. These peripheral blood progenitor cells (PBPC) may be harvested and infused after high-dose cytotoxic therapy, either in place of or in addition to bone marrow transplantation.

G-CSF drug products, such as ZARXIO and Neupogen, share the same pharmacology and thus are expected to have no meaningful differences in clinical effects across the range of approved indications. Through the demonstration of analytical similarity in the ZARXIO development program, coupled with the confirmation of similarity in the comprehensive nonclinical and clinical evaluation, the extension to all indications for which the reference medicinal product Neupogen is approved is justified.

ZARXIO is to be administered subcutaneously or intravenously and will be provided in pre-filled syringes (PFS) containing 300 mcg/0.5 mL or 480 mcg/0.8 mL. The PFS are similar to that of the reference product (Neupogen).

ZARXIO was approved first in the EU in 2009 and is marketed in most countries of the European Economic Area (as Zarzio) as well as in 32 additional countries worldwide. All approvals have included all indications of Neupogen. This market experience, with more than 7.5 million patient days of product exposure, provides further confirmation of the product's utility and safety and demonstrates no meaningful differences compared to the reference product's prescribing information and clinical history. The remaining sections of the briefing book provide details of the development program supporting the similarity of ZARXIO to the reference product Neupogen.

Biosimilars in general, and those of filgrastim specifically, are expected to yield significant public health advantages. Not only can their availability increase patient access to life saving medicines and enhance security of supply, but they will likely also offer savings for healthcare systems as has already been seen in Europe.

While biologics have had tremendous impact on medicine, there are limited therapeutic alternatives for many of these biologic medicines. By introducing greater competition into an area that has historically been devoid of it, biosimilar medicines offer more choices to healthcare providers and patients, thereby increasing patient access without increasing overall spending. This is particularly important in oncology care, as the cost of cancer treatment accounts for a large portion of health care spending, and this spending is only projected to increase. Furthermore, savings generated may then be used to fund medical innovation that addresses unmet needs.

2.2.1 Reference product

The single reference product in the BLA for ZARXIO is US-licensed Neupogen. To establish the critical quality attributes of the reference product, Sandoz conducted a comprehensive analysis of over 80 batches of Neupogen, including multiple time points during the shelf-life studies.

The pivotal data supporting biosimilarity make a direct comparison between ZARXIO and US-licensed Neupogen. EU-authorized Neupogen was also evaluated as part of ZARXIO development. While our extensive analytical comparisons of Neupogen sourced from the US and EU did not reveal differences (data not shown), we have noted throughout this briefing document when the EU material was specifically studied.

The ZARXIO formulation is identical to that used for Neupogen in the US with the exception of the buffer used – a glutamate buffer is used to control the pH instead of an acetate buffer (see Section 3.4).

2.2.2 Indications

The table below reviews the proposed indications for ZARXIO and compares them to the currently approved indications for Neupogen in the US and Europe (and Zarzio in Europe). The proposed indications for ZARXIO are identical to those of US-licensed Neupogen.

Table 1 - Indications

US-licensed Neupogen	**Proposed indications for ZARXIO in US**	**EU-authorized Neupogen**	**EU-authorized ZARXIO (trade name: Zarzio)**
Cancer patients receiving myelosuppressive chemotherapy	*Cancer patients receiving myelosuppressive chemotherapy*	*Patients treated with established cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes)*	*Patients treated with established cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes)*

US-licensed Neupogen	Proposed indications for ZARXIO in US	EU-authorized Neupogen	EU-authorized ZARXIO (trade name: Zarzio)
Patients with acute myeloid leukemia receiving induction or consolidation chemotherapy	*Patients with acute myeloid leukemia receiving induction or consolidation chemotherapy*	*Included in the indication above*	*Included in the indication above*
Cancer patients receiving bone marrow transplant	*Cancer patients receiving bone marrow transplant*	*Patients undergoing myeloablative therapy followed by bone marrow transplantation considered to be at increased risk of prolonged severe neutropenia*	*Patients undergoing myeloablative therapy followed by bone marrow transplantation considered to be at increased risk of prolonged severe neutropenia*
Patients undergoing peripheral blood progenitor cell collection and therapy	*Patients undergoing peripheral blood progenitor cell collection and therapy*	*Mobilization of peripheral blood progenitor cells (PBPC) in patients undergoing myelosuppresive or myeloablative therapy and in normal donors*	*Mobilization of peripheral blood progenitor cells (PBPC)*
Patients with severe chronic neutropenia	*Patients with severe chronic neutropenia*	*Patients with severe congenital, cyclic, or idiopathic chronic neutropenia*	*Patients with severe congenital, cyclic, or idiopathic chronic neutropenia*
-	-	*Treatment of persistent neutropenia in patients with advanced HIV infection*	*Treatment of persistent neutropenia in patients with advanced HIV infection*

2.2.3 Mechanism of action – filgrastim

The mechanism of action of filgrastim in neutropenia is well understood, and treatment with G-CSF products in the different neutropenia indications is mediated *via* a single mechanism of action (Bugl 2012, Wirths 2013).

Filgrastim selectively and specifically stimulates the proliferation and differentiation of neutrophil precursors through binding to its receptor (G-CSFR) (

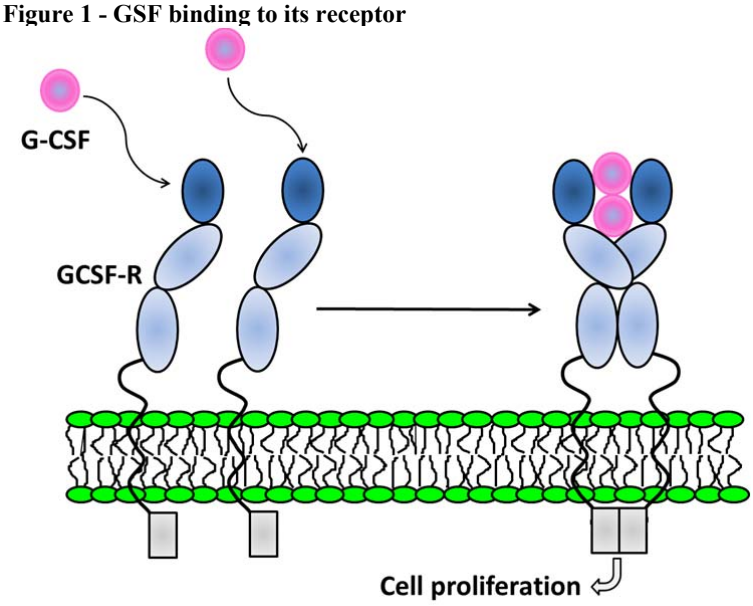
Figure 1). G-CSFR-induced signaling is required throughout the development of the neutrophil lineage, beginning with myeloid-committed progenitors. It is also vital for regulating terminal neutrophil differentiation, since the residual neutrophils found in G-CSFR$^{-/-}$ animals exhibited defective functional responses, including impaired adhesion and chemo-attractant-induced migration. In addition to elevating neutrophil levels, G-CSF also reduces the neutrophil maturation time, leading to a rapid release of neutrophils from the marrow into the circulation (Welte 1996, Panopoulos 2008).

Compared to neutrophil proliferation, the mechanism of action of filgrastim in the release of hematopoietic progenitor cells from their bone marrow niches is not quite as well understood. Hematopoietic progenitor cells generally express the G-CSF receptor, and data in G-CSF receptor deficient mice showed that the complete absence of the G-CSF receptor in the animal precludes an increase in the level of circulating hematopoietic progenitor cells (Link 2000). This confirms that the mobilization of hematopoietic progenitor cells is also a G-CSF receptor-mediated process.

Nonetheless, it is clear is that the filgrastim-induced increase in neutrophil maturation and mobilization in neutropenia and the mobilization of hematopoietic progenitor cells are both the endpoint of a cascade of events that are initiated by selective binding of filgrastim to the G-CSF receptor.

Accordingly, the demonstration of the highly similar molecular structure and function confirmed by clinical effectiveness in one sensitive indication, for instance in the treatment of neutropenia in cancer patients receiving myelosuppressive chemotherapy, would be expected to be indicative of benefit in other indications where G-CSF is known to be effective. Our data also show highly similar mobilization of CD34^{+} cells into the peripheral blood.

Figure 1 - GSF binding to its receptor



3 ZARXIO product development - designed to match the reference product

ZARXIO was systematically developed to be highly similar to its reference product Neupogen, i.e. to be highly similar to Neupogen regarding the active pharmaceutical ingredient (API), formulation and finished product.

The ZARXIO manufacturing process consists of the following main steps:

Active pharmaceutical ingredient:

- Fermentation starting with the cell line
- Isolation and purification

Finished product:

- Compounding (formulating)
- Filling into syringes
- Packaging

Below, we provide descriptions as to how the manufacturing process was developed at each of these steps to result in a product that is highly similar to Neupogen.

3.1 Development of cell line

As is the case with Neupogen, the ZARXIO API is expressed in *E. coli*, which is a well-known host used in the manufacture of recombinant proteins.

The *E. coli* cells were transformed with a plasmid carrying an optimized, partially synthetic, structural gene for r-metHuG-CSF. The gene was designed to optimize precision in protein biosynthesis and lead to equal or lower levels of product variants compared to Neupogen. This gene is integrated into an expression plasmid which uses well established and safe components. The expression plasmid is then introduced into an *E. coli* strain, which has been widely and safely used in the biopharmaceutical industry for many years.

A large number of clones resulting from this so-called transformation are then screened to identify the production clone – a clone which combines high quality and robust productivity. The selection process included purification and characterization of G-CSF from each clone. Only clones that yielded product that closely matched the reference product were pursued for continued development. The final clone selected for full development was extensively tested for safety and genetic stability and was preserved in a Master Cell Bank and a Working Cell Bank. These cell banks contain many hundred vials each and are stored at ultra-low temperatures in containers cooled with liquid nitrogen. This ensures that the cell line does not change over time and that a consistent starting point for manufacturing is available over the entire product lifetime.

3.2 Development of the fermentation process

To initiate the fermentation process, a vial of the Working Cell Bank is thawed under controlled conditions and its contents are transferred into a shake flask containing a sterile nutrient medium. The cells are grown in this shake flask and then transferred to bioreactors (fermenters) of increasing size until the large, main-stage fermenter is reached.

All of the steps in the fermentation process were optimized to yield a product quality that is highly similar to Neupogen. At all stages, nutrient media without any animal-derived components are used to avoid the risk of contamination with such components. The cultivation conditions were systematically optimized in an iterative manner with many fermentation runs, manipulating the culture conditions until we were able to achieve the required product quality that was highly similar to the reference product. This included the optimization of the components of the nutrient medium to provide optimal supply to the cells at all times and the optimization of cultivation conditions such as temperature, stirring speed, air input, and pH.

3.3 Development of the isolation and purification process

As the product accumulates inside of the cells, in inclusion bodies, a process for lysing the cells was developed. This is achieved by exerting the cells to high pressure and then rapidly releasing this pressure using a high pressure homogenizer. This process is very effective, yet gentle, and was optimized to ensure the API is not damaged in any way. The inclusion bodies are then isolated from the cell debris using a special centrifuge (separator) and washed thoroughly to remove components of the *E. coli* cell.

As the inclusion bodies are an insoluble form of the API, they must be dissolved ("denatured") and then brought into the right folding configuration ("refolded"). This is achieved in a carefully developed process involving a well-established denaturing agent and conditions optimized to yield the protein in its correctly folded state. Special attention is paid to achieving the correct folding, and the formation of the correct disulphide bonds while maintaining the integrity of the amino acid side chains.

Starting from this material, the purification process was then developed. The goal was to achieve an API that contains equal or lower levels of product variants compared to Neupogen. Another goal was that it contains extremely low levels of *E. coli* proteins. Multiple purification steps were evaluated, modified and refined in an iterative manner to yield product quality that was highly similar to the reference product. The process selected contained a series of sequential chromatography and filtration steps that use complementary purification methods, ensuring that the latter steps reduce residual impurities that may be present. In the end, the resulting API has very low levels of variants at or below the levels found in Neupogen, and extremely low levels of *E. coli* proteins (less than 50 ppm (ng/mg), or 0.00005%).

The API is frozen in special sterile plastic bottles until needed for the manufacturing of finished product.

3.4 Development of the formulation and the finished product

As was done for the drug substance or API, the formulation was developed to closely match the properties of the Neupogen formulation. The ZARXIO formulation is identical to that used for Neupogen in the US with the exception of the buffer used – a glutamate buffer is used to control the pH of ZARXIO instead of an acetate buffer. This small difference was the result of a patent issue. The ZARXIO formulation was, however, developed to be very close to Neupogen with respect to all relevant pharmaceutical parameters, such as buffer strength, pH, osmolality and contained surfactant. It can therefore be considered pharmaceutically highly similar. Of note, the BPCIA specifies that different formulations are permissible as long as there is no impact on safety or clinical efficacy.

The ZARXIO finished product presentation is of the same nature as the Neupogen finished product – a prefilled syringe with a ready-to-use solution for injection. The API, filgrastim, is formulated in a clear, colorless, preservative-free solution at a concentration of 0.6 mg/mL provided in 1 ml PFS with 0.5 mL or 0.8 mL as outlined in

Table 2, below. Concentration (strength) and filling volumes match those of Neupogen.

Table 2 - Comparison of composition (ZARXIO vs. Neupogen) – 600 mg/mL

Function	**ZARXIO**		**Neupogen**	
Active Ingredient	**Ingredient**		**Ingredient**	
API	Filgrastim	0.600 mg/mL	Filgrastim	0.600 mg/mL
Other Ingredients*				
Buffer	Glutamate	10 mM	Acetate	10 mM
Tonifying agent	Sorbitol	50 mg/mL	Sorbitol	50 mg/mL
Surfactant	Polysorbate 80	0.004%	Polysorbate 80	0.004%
Solvent	Water for Injection	ad 0.5 mL or 0.8 mL	Water for Injection	ad 0.5 mL or 0.8 mL
pH	4.4		4.0	

*ZARXIO uses sodium hydroxide for pH adjustment

In summary, ZARXIO was systematically developed to closely match Neupogen regarding both the finished product and the API contained therein. ZARXIO contains levels of variants that are equal to or less than the levels found in Neupogen. The formulation is pharmaceutically highly similar to Neupogen and both products are provided in the same strengths in the same kind of prefilled syringe.

Since initial registration in 2009, scale-up and transfer of drug substance and drug product manufacturing have occurred to meet increased demand. Comparability exercises in compliance with the ICH guideline Q5E were performed for all such transfers. These have all been evaluated and approved by the European Medicines Agency, including the sites intended to supply the US with drug product. An overview on the drug product manufacturing sites is

provided in Table 3. Drug substance (the API) for the US is manufactured by Sandoz GmbH, Austria.

The pivotal clinical PK/PD study, Study 109, comparing US-licensed Neupogen to ZARXIO was conducted with ZARXIO in pre-filled syringes. The pivotal clinical safety and efficacy confirmation study, Study 302, comparing ZARXIO to US-licensed Neupogen was conducted with ZARXIO vials. The identity, strength (where applicable), purity, potency and molecular attributes of ZARXIO in vials and PFS were analyzed extensively and found to be comparable. A comparability study comparing US and EU-sourced Neupogen was also performed to establish an analytical bridge between the drug products used in the pivotal US clinical studies and supportive studies carried out in the EU. The drug substance and pre-filled syringes manufactured for the EU and US markets were found to be comparable, providing an additional bridge to the supportive European studies.

Table 3 - Overview on Drug Product Manufacturing sites and use of drug product

Use	Drug Product Manufacturing site
European PK/PD studies	Lek Pharmaceuticals, d.d, Slovenia, a subsidiary of Sandoz
European Safety study	Lek Pharmaceuticals, d.d, Slovenia, a subsidiary of Sandoz
European PK/PD study and US PK/PD study	IDT Biologika, Germany
US safety study	Novartis Pharma, Switzerland
ZARXIO intended for US market	GP Grenzach GmbH, Germany

3.5 Regulatory history and FDA interactions

ZARXIO was initially authorized in Europe according to the guidelines for biosimilar medicinal products in 2009 (marketed as Zarzio). Based on the demonstration of similarity and the principle of extrapolation, all indications approved for Neupogen in Europe were also approved for Zarzio (see Section 5 for the rationale for extrapolation of all indications).

Sandoz has interacted with FDA to discuss and agree on the development program and data package necessary for licensure of ZARXIO as a biosimilar in the US. The primary FDA feedback and agreements were:

- The content of the data package must be sufficient to demonstrate analytical similarity.
- Acceptability of use of a different buffer than that used in Neupogen if it does not introduce clinically meaningful differences (Note that this is consistent with BPCI Act, which states that the requirement for analytical high similarity to the reference product is “notwithstanding minor differences in clinically inactive components.”)
- Similarity could be demonstrated with a single phase I PK/PD study directly comparing ZARXIO to US-licensed Neupogen. Study 109 was conducted to fulfill this FDA requirement.

- Similarity could be confirmed with a single "*phase III trial involving a direct comparison of ZARXIO with US-licensed Neupogen to demonstrate safety, purity, and potency"* Consequently, Study 302 was designed and the protocol was provided to FDA for review and approval prior to study initiation. This study demonstrated the same clinical performance (non-inferiority).
- Extrapolation of indications is justifiable; provided *"sufficient scientific justification for extrapolating clinical data to support a determination of biosimilarity for each condition of use for which you seek licensure"*. The requested justification was provided in the BLA.

Sandoz considers that the data and results presented in the BLA fulfill the statutory requirements for the licensure of a biological product as biosimilar and are consistent with the guidance and agreement received from the FDA in the development of ZARXIO (please also refer to Section 6)

3.6 Post-marketing experience

ZARXIO (as Zarzio) is licensed in all countries of the European Economic Area (the European Union, Norway, Iceland and Liechtenstein) and in an additional 32 countries worldwide. Post-marketing experience since February 2009 represents in excess of 7.5 million patient treatment days of therapy, 7 million of which were obtained in countries of the European Economic Area. The results from the pharmacovigilance of the post-marketing experience are provided in Section 8.5.3 of this document and the safety profile of ZARXIO remains in line with the previous cumulative experience and safety information available for Neupogen.

4 Concept of biosimilarity

4.1 Regulatory definition of a biosimilar medicine

A biosimilar is a biological product that is highly similar to an already approved biological product, notwithstanding minor differences in clinically inactive components, and for which there are no clinically meaningful differences between the biosimilar and the approved biological product in terms of the safety, purity, and potency (Biologics Price Competition and Innovation Act of 2009]).

4.2 Regulatory paradigm/construct for demonstrating biosimilarity

The Patient Protection and Affordable Care Act (ACA), signed into law on March 23, 2010 created a licensure pathway for biological products that are demonstrated to be "biosimilar" to or "interchangeable" with an FDA-licensed biological product.

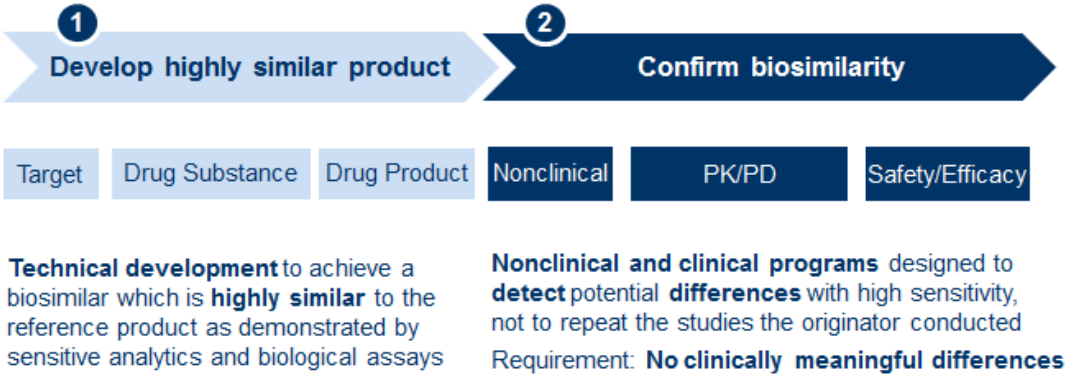
Demonstration of biosimilarity is based on the "totality-of-evidence" concept. That is, FDA has emphasized that similarity with respect to a specific property or area of testing (e.g. physicochemical, biological, nonclinical or clinical) is not sufficient in isolation to establish similarity, but rather a comprehensive stepwise evaluation of multiple lines of evidence is necessary for concluding that a proposed biosimilar is approvable.

4.3 Stepwise approach to the development of biosimilar drugs

A "stepwise approach" is essential to develop a biosimilar drug and requires a detailed understanding of the structure and function of the reference drug, as the first step is to develop a highly similar product which closely matches the reference drug substance and drug product. This step requires detailed evaluation of the reference drug as well as innovative techniques for the development, production and characterization of the similar product. Specifically, it starts with the physicochemical characterization, followed by biological characterization and is completed with confirmatory preclinical and clinical evaluations. In each of these phases, the goal is to gain additional support for the establishment of biosimilarity and to remove any residual uncertainty that the product will perform as expected.

This approach and the various stages are depicted in Figure 2.

Figure 2 - The two development stages for biosimilars



5 Extrapolation of indications

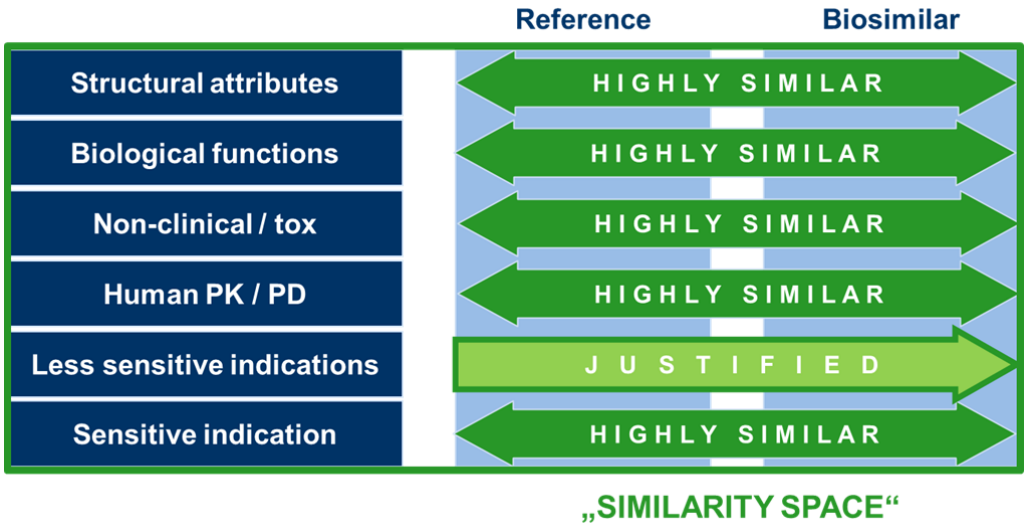
This application seeks licensure for all indications for which the reference product, Neupogen, is approved. This is similar to the case in Europe, where Zarzio was granted all indications for which Neupogen is approved, based on the totality of the data.

The rationale for extrapolation is based on the understanding that once similarity of products is rigorously and unambiguously established, biosimilarity applies to the entire clinical experience generated with the reference product for all indications, including the clinical studies conducted in all indications as well as from the real-life-experience accumulated through the extensive use of the reference product in the various indications and patient populations.

Extrapolation includes not only efficacy data but safety data as well, with the caveat that immunogenicity must be studied separately for each biosimilar. Furthermore, data established with the originator in special populations such as ethnicity, sex and age are also extrapolated from the originator to the biosimilar. Since studies with the reference product have established that no differences have been detected between different ethnic groups, similar studies do not need to be repeated with ZARXIO.

Importantly, efficacy and safety of a biosimilar is not extrapolated from one indication to another. Rather, extrapolation is based upon the understanding that if the biosimilar product has been demonstrated to be highly similar to the reference product through multiple lines of evidence, then it is expected to have similar clinical activity in all clinical settings for which the reference product has been tested and approved. This is shown graphically in the following scheme.

Figure 3 - Concept of extrapolation in the context of biosimilarity



Based on the totality of evidence demonstrating high similarity at all of levels, it can be concluded that the biosimilar will elicit the same response as the reference drug in all indications as was demonstrated in the most sensitive indication. Thus it is clear that

extrapolation is from the efficacy and safety of the reference product to the biosimilar within each indication, and is not an extrapolation of the efficacy and safety of the biosimilar from one indication to another.

The scientific principles underlying extrapolation were recently described in a scientific article published by members of the EMA Biosimilars Medicinal Products Working Party (Weise 2014):

> *"From a scientific and regulatory point of view, the active substance of the biosimilar is just another version of the active substance of the originator product. This is important to state since the same scientific principles that underlie the comparability exercise for the purpose of demonstrating similarity of a product before and after a change in manufacturing process also apply to the comparability exercise for the purpose of demonstrating biosimilarity. The cornerstone of any such comparability exercise is the extensive comparison of the physicochemical and functional characteristics of the molecules (e.g. molecular structure including glycosylation, receptor binding, biological activity) using up-to-date analytical tools."*

The authors of this paper also specifically address extrapolation of indications for filgrastim biosimilars that were demonstrated to be similar to the reference product:

> *"All pharmacological actions of filgrastim are mediated via a single affinity class cell receptor. Therefore, comparable receptor binding as demonstrated for the biosimilar and the reference filgrastims is expected to result in comparable downstream effects, regardless of potential differences in target cell-specific intracellular signalling pathways, e.g. in hematopoietic progenitor cells vs. mature neutrophils.*
>
> *Unsurprisingly, postmarketing studies confirmed efficacy and safety of biosimilar filgrastim products in the approved indications including mobilization of stem cells in healthy donors [29-34]."*

In addition, for those rare cases in which clinical studies are required as part of a comparability assessment following a manufacturing change for a licensed product, they are almost never required for more than one clinical indication for which the product is licensed.

In summary, justification for the extrapolation of all indications is based on:

1. Demonstration of analytical and functional similarity between ZARXIO and Neupogen; consequently the two products can be expected to act in the same way in all patient populations.
2. A single mode of action across all indications for filgrastim, i.e. binding specifically to the G-CSF receptor.
3. The presence of clinical data that demonstrate the pharmacokinetic and pharmacodynamic equivalence of ZARXIO and Neupogen in healthy volunteers, including the mobilization of $CD34^+$ cells into the peripheral blood.
4. Demonstrated effectiveness in the prophylactic treatment of neutropenia in cancer patients undergoing myelosuppressive chemotherapy.

6 Fulfillment of statutory requirements

Multiple lines of evidence are presented in the ZARXIO BLA to satisfy the statutory definition of biosimilarity. Table 4 below, reviews the statutory requirements for establishing biosimilarity and provides a brief explanation of how the BLA contents fulfill the requirements and contribute to the totality of scientific evidence establishing the biosimilarity of ZARXIO to Neupogen.

Table 4 - Point-by-point summary of ZARXIO BLA's fulfillment of statutory requirements

Statutory requirement	Statute language	ZARXIO BLA fulfillment of requirement
Reference product	*351(k)(5)(A)* *One reference product per application. A biological product, in an application submitted under this subsection, may not be evaluated against more than 1 reference product*	The single reference product in the BLA for ZARXIO is US-licensed Neupogen.
Analytical data	*351(k)(2)(A)(i)(I)(aa)* *Analytical studies that demonstrate that the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components*	The analytical data (presented in Section 7.1) demonstrate that ZARXIO is highly similar to the reference product from an analytical and functional standpoint including: primary and higher order structure (incl. molecular mass, size, charge, hydrophobicity, etc.), function, purity, stability etc. as well as bioactivity.
Animal studies	*351(k)(2)(A)(i)(I)(bb)* *Animal studies (including the assessment of toxicity)*	ZARXIO was compared with Neupogen in five animal studies assessing pharmacodynamics, toxicity, toxicokinetics, and local tolerance. Nonclinical results confirmed that the pharmacologic and toxicologic profiles of ZARXIO and Neupogen are similar.
Clinical studies	*351(k)(2)(A)(i)(I)(cc)* *A clinical study or studies (including the assessment of immunogenicity and pharmacokinetics or pharmacodynamics) that are sufficient to demonstrate safety, purity, and potency in 1 or more appropriate conditions of use for which the reference product is licensed and intended to be used and for which licensure is sought for the biological product*	Clinical studies were conducted to assess immunogenicity, pharmacokinetics, and pharmacodynamics, as well as clinical efficacy and safety of ZARXIO. Relevant clinical data were collected in a total of 174 healthy volunteers, 388 breast cancer patients receiving myelosuppressive chemotherapy, and 121 healthy stem cell donors.
Mechanism of action	*351(k)(2)(A)(i)(II)* *The biological product and reference product utilize the same mechanism or mechanisms of action for the condition or conditions of use prescribed, recommended, or suggested in the proposed labeling, but only to the extent the mechanism or mechanisms of action are*	The mechanism of action of filgrastim as an rhG-CSF product is mediated by the selective binding to the G-CSF receptor and is similar across all indications. There are no known qualitative differences in the mechanism of action in neutropenia of different origins.

Statutory requirement	Statute language	ZARXIO BLA fulfillment of requirement
	known for the reference product	
Conditions of use	*351(k)(2)(A)(i)(III)* *The condition or conditions of use prescribed, recommended, or suggested in the labeling proposed for the biological product have been previously approved for the reference product*	The ZARXIO BLA submission seeks licensure for the same indications for which the reference product is approved. (See Section 2.2)
Route of administration, dosage form, and strength	*351(k)(2)(A)(i)(IV)* *The route of administration, the dosage form, and the strength of the biological product are the same as those of the reference product*	ZARXIO has the same route of administration, dosage form, and strengths as the reference product.
Fulfillment of the definition of "biosimilar"	*351(k)* *''(2) The term 'biosimilar' or 'biosimilarity', in reference to a biological product that is the subject of an application under subsection (k), means—*	
	''(A) that the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components; and	(A) Section 7.1 of this briefing book describes the high analytical and functional similarity of ZARXIO and Neupogen
	''(B) there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product.	(B) The data presented in Sections 7.3 establish that there are no clinically meaningful differences between ZARXIO and Neupogen.

7 Demonstration of biosimilarity

7.1 Analytical demonstration of biosimilarity

Demonstration of similarity to the reference product using powerful analytical tools is a fundamental step in establishing biosimilarity and is driven by an understanding of the structure of the target molecule and its biologic functioning.

7.1.1 Link between structure, function, and physiological response

As noted previously, the mechanism of action of G-CSF is mediated through its binding to its receptor (G-CSFR). (Please see Section 2.2.3 of this document.) This unique receptor is highly expressed on blood cells of the neutrophilic granulocyte lineage. Binding of G-CSF causes a dimerization of the G-CSFR and activates downstream signaling cascades. Activation of the downstream signaling is driven by the structural integrity of the binding interface between G-CSF and its receptor.

The interaction between G-CSF and G-CSFR is well characterized on a molecular level based on x-ray structure of the G-CSF/G-CSFR complex and mutagenesis analysis.

The structure and function of filgrastim determines the *in vivo* physiological response, and must therefore be controlled. Critical molecular attributes (commonly termed as "quality attributes") that are responsible for the *in vivo* physiological response to filgrastim are the following:

- the **amino acid sequence (identity)** for driving correct folding and providing the amino acid side-chains for the G-CSF/G-CSFR interaction
- **chemical modifications of amino acids (purity), in particular methionine oxidation** which lead to a structural and functional perturbation of the binding sites
- **high-molecular weight variants/aggregates (purity)**
- the overall **three-dimensional structure (higher order structure)** which serves as the scaffold for correct positioning of the binding sites
- **receptor binding** reflecting the integrity of the overall structure and the binding sites
- ***in vitro* proliferation assay (potency)** for demonstrating activation of downstream signaling cascades as a result of full structural integrity of the molecule. The assay mimics the mode of action in the clinical setting
- **protein concentration (content**) for correct dosing of the drug

In designing an analytical analysis program, emphasis was placed on evaluating the quality attributes that are known to have clinical relevance, as outlined in Table 5 below. The color coding highlights the relative importance of the various quality attributes; with the elements highlighted in red being most important and essential for demonstrating similarity as well as safety and effectiveness. The relative importance was assigned by Sandoz based on literature knowledge and our own targeted experiments in which the quality attributes were varied and assessed (data not shown).

Table 5 - Criticality of quality attributes and their impact on clinical parameters

Quality Attribute	Criticality	Relevant for	Methods Used
Amino acid sequence	Very High	Efficacy, Safety, Immunogenicity	Edman, peptide mapping, MS
Potency	Very High	Efficacy Safety	Bioassay
Target binding	Very High	Efficacy Safety	Surface plasmon resonance
Protein concentration	Very High	Efficacy	Content determination
Subvisible particles	High	Immunogenicity	Light obscuration
Oxidized variants	High	Efficacy	Reversed phase chromatography
Higher order structure	High	Efficacy Immunogenicity	CD and NMR spectroscopy
High-molecular weight variants/aggregates	High	Immunogenicity	Size exclusion chromatography
Truncated variants	Low	None	Reversed phase chromatography coupled with MS
Norleucine	Very Low	None	Reversed phase chromatography
Deamidation	Very Low	None	Cation exchange chromatography

The final head-to-head analytical comparison was performed comparing six batches of ZARXIO with four batches of US-licensed Neupogen and two batches of EU-authorized Neupogen, but more than 80 batches of Neupogen and ZARXIO were analyzed and compared in the course of development. Given the extensive database, only representative data and their associated methods are provided for ease of review.

7.1.2 Analytical comparison between ZARXIO and Neupogen

The analytical comparison of ZARXIO to the reference product was conducted in a stepwise manner that assessed structure, function, potential variants and stability. The comparative analyses were conducted with drug product.

Analytical comparison begins with a comparison of the structure of the biosimilar with reference product. The amino acid sequence and protein folding have to match.

Once the structure is demonstrated to be highly similar, functional assays are conducted to establish that receptor binding properties and potency are also highly similar. For filgrastim, potency was assessed by use of an *in vitro* myeloid cell proliferation assay.

Further comparisons are conducted to evaluate potential variations in size and amino acid side chain structure.

Once high similarity was established with ZARXIO and Neupogen, further analyses were conducted to establish that ZARXIO exhibits the same product stability profile as is observed with Neupogen.

7.1.3 Structural assays

7.1.3.1 The primary structure of ZARXIO and Neupogen are identical

The primary structure of a protein is the sequence of amino acids. Having the same amino acid sequence is important for safety, including immunogenicity, and efficacy. Confirmation that ZARXIO has the same amino acid sequence as Neupogen was demonstrated by multiple complementary methods, including classical N-terminal Edman sequencing and sequencing by tandem mass spectrometry (LC-MS/MS).

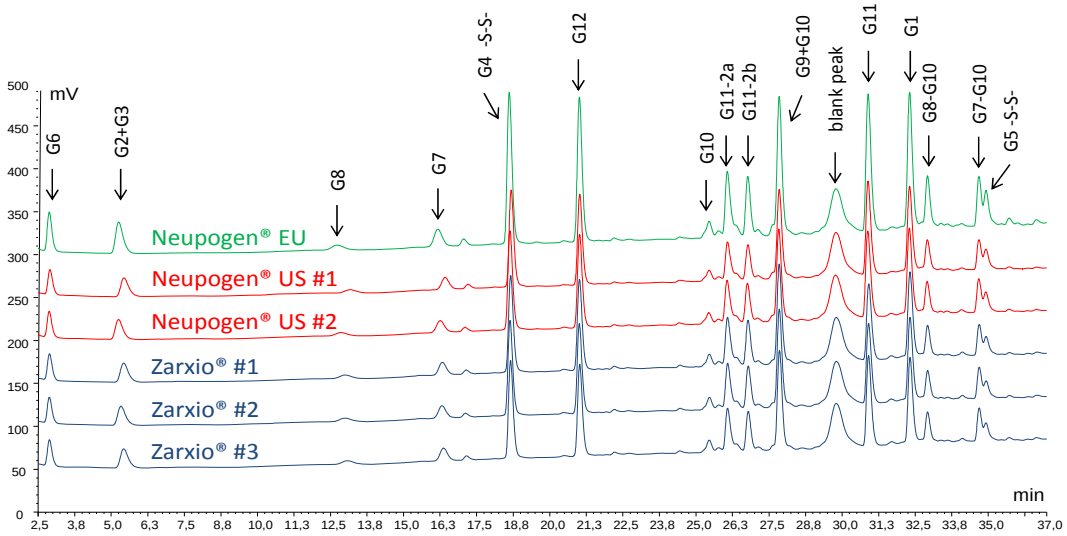
Figure 4 shows the sequence alignment of the determined amino acid sequences of ZARXIO and Neupogen Both, ZARXIO and Neupogen have identical amino acid sequences.

Figure 4 - Sequence alignment between ZARXIO and Neupogen

ZARXIO	MTPLGPASSLPQSFLLKCLEQVRKIQGDGAALQEKLCATYKLCHPEELVLLGHSLGIPWAPLSSCPSQALQL
Neupogen®	MTPLGPASSLPQSFLLKCLEQVRKIQGDGAALQEKLCATYKLCHPEELVLLGHSLGIPWAPLSSCPSQALQL
ZARXIO	AGCLSQLHSGLFLYQGLLQALEGISPELGPTLDTLQLDVADFATTIWQQMEELGMAPALQPTQGAMPAFASA
Neupogen®	AGCLSQLHSGLFLYQGLLQALEGISPELGPTLDTLQLDVADFATTIWQQMEELGMAPALQPTQGAMPAFASA
ZARXIO	FQRRAGGVLVASHLQSFLEVSYRVLRHLAQP
Neupogen®	FQRRAGGVLVASHLQSFLEVSYRVLRHLAQP

In addition to the full sequencing described above, peptide mapping analysis with UV and mass spectrometric detection were performed to compare the amino acid sequence of ZARXIO and Neupogen and confirm that the sequences are identical. In this analytical assay the protein is digested to smaller fragments using a specific protease and the fragments are subsequently separated by reversed-phase high-performance liquid chromatography (RP-HPLC). Because the digestion is performed under non-reducing conditions the methods also allows assaying for correct disulfide-bond formation. Figure 5 shows an overlay of the RP-HPLC chromatograms of a Glu-C digest peptide map from ZARXIO and Neupogen demonstrating identical amino acid sequence and disulfide-bond formation. Mass spectrometry confirms the identity of each peak by measuring the mass of the generated peptide fragments.

Figure 5 - Overlay of RP-HPLC chromatograms of a Glu-C digest peptide map from ZARXIO and Neupogen



7.1.3.2 Folding: Three-dimensional structure (higher order structure) does not differ between ZARXIO and Neupogen

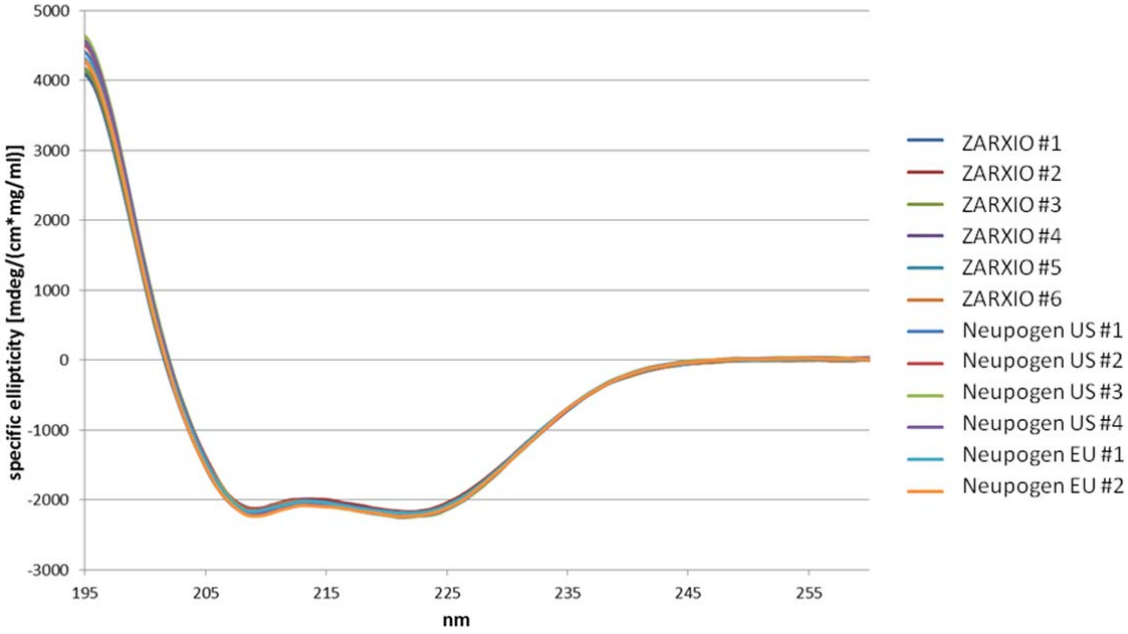
The three-dimensional structure of filgrastim drives its function by defining the interactions with the G-CSF receptor. Engagement with the receptor activates the downstream signaling cascades that culminate in the biological activities of G-CSF, including myeloid cell proliferation, differentiation, and activation. The three-dimensional structure is therefore responsible for the efficacy of the molecule. It is also relevant to immunogenicity as incorrectly folded proteins may cause immune reactions.

To demonstrate high similarity with regard to secondary and tertiary structure Sandoz has used circular dichroism spectroscopy (CD), 1D-[1H]-NMR and 2D-[1H-15N] HSQC (heteronuclear single quantum coherence) NMR spectroscopy. CD analyzes folding at the level of the secondary structure, *i.e.* the coiling of the strand of amino acids into alpha helices or its organization into beta sheets. NMR investigates folding of the protein as a whole by directly comparing the relative positions of the individual amino acids in each of the two products.

For all tested ZARXIO and Neupogen batches, the far UV CD spectra indicate a folded protein with high amounts of α-helical secondary structure composition, with characteristic minima at 208 nm and 222 nm. Transition points and the ratios of specific ellipticity (θR)208nm/(θR)222nm were highly similar between ZARXIO and Neupogen.

Figure 6 depicts an overlay of the CD spectra acquired in the head-to-head biosimilarity study.

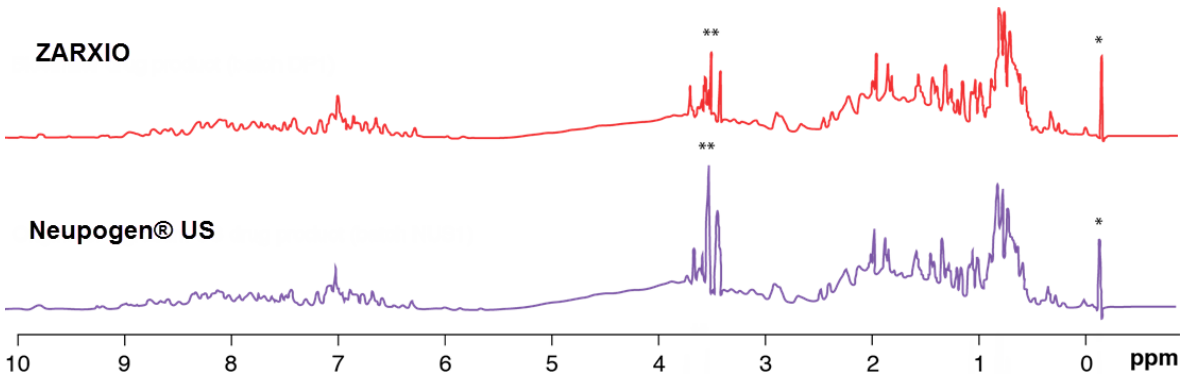
Figure 6 – Far UV CD spectra of ZARXIO and Neupogen batches



CD spectra of six ZARXIO batches, four US-licensed Neupogen US and two EU-authorized Neupogen batches demonstrating a high degree of structural similarity.

Nuclear magnetic resonance (NMR) spectroscopy was used to further demonstrate the structural high similarity between ZARXIO and Neupogen. Figure 7 shows a representative overlay of the 1D-{1H}-NMR spectrum of one batch of ZARXIO and one batch Neupogen. All samples exhibited NMR signal dispersions between 10.0 and 0.0 ppm indicating fully folded proteins with a high content of α-helical secondary structure elements. No significant differences were detected between ZARXIO and Neupogen.

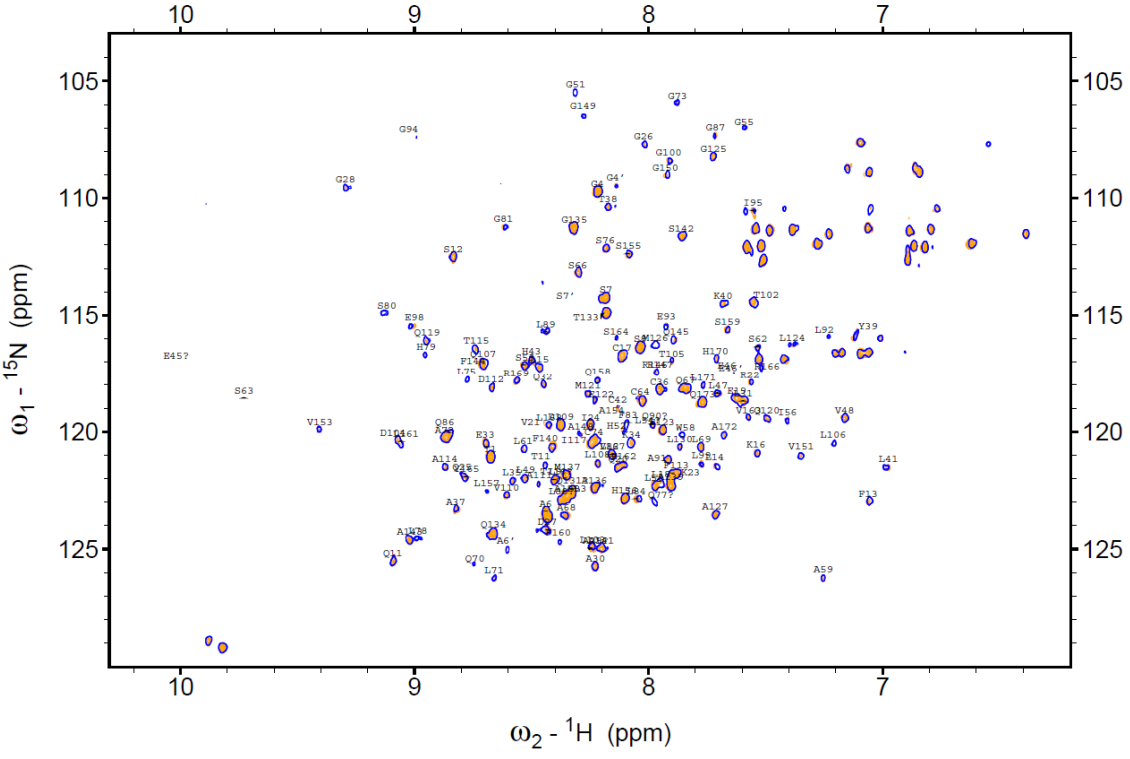
Figure 7 – 1D-{1H}-NMR spectrum of one representative batch of ZARXIO and Neupogen



1D-{1H}-nuclear magnetic resonance (NMR) spectra of ZARXIO, Neupogen US and Neupogen EU. * indicates d4-TSP signal; ** signals between 3.4 and 3.9 ppm correspond to formulation components, i.e. are not protein related. d4-TSP = 2,2,3,3-d4 sodium 3-(trimethylsilyl)propionate.

Amide region ^{1}H-^{15}N HSQC (heteronuclear single quantum coherence) fingerprints (2D-NMR fingerprint) offers enhanced capabilities compared to 1D-{1H}-NMR spectroscopy with resolution down to the single amino acid level. Figure 8 illustrates a representative overlay of the 2D-NMR spectra of one batch of ZARXIO and one batch of Neupogen. The analysis shows a high degree of structural similarity between ZARXIO and Neupogen considering peak shifts in both ^{1}H-^{15}N HSQC coordinates along with spectral information such as line-width and signal to noise ratio.

Figure 8 – 2D-NMR fingerprint of one representative batch each of ZARXIO and Neupogen



Overlay of amide region ^{1}H-^{15}N HSQC (heteronuclear single quantum coherence) fingerprints of one US-licensed Neupogen batch (orange) and one ZARXIO batch (blue) demonstrating a high degree of structural similarity. Because the spots overlap entirely, in order to better visualize the results, the contour levels in the ZARXIO spectrum (blue) were adjusted so that only the outline is visible in the spectrum.

In summary the data from CD, 1D-[1H]-NMR and 2D-[1H-15N] HSQC NMR spectroscopy demonstrate the high similarity between ZARXIO and Neupogen with respect to the three-dimensional structure (folding) of filgrastim.

7.1.4 Functional assays

7.1.4.1 Target binding of ZARXIO and Neupogen are highly similar

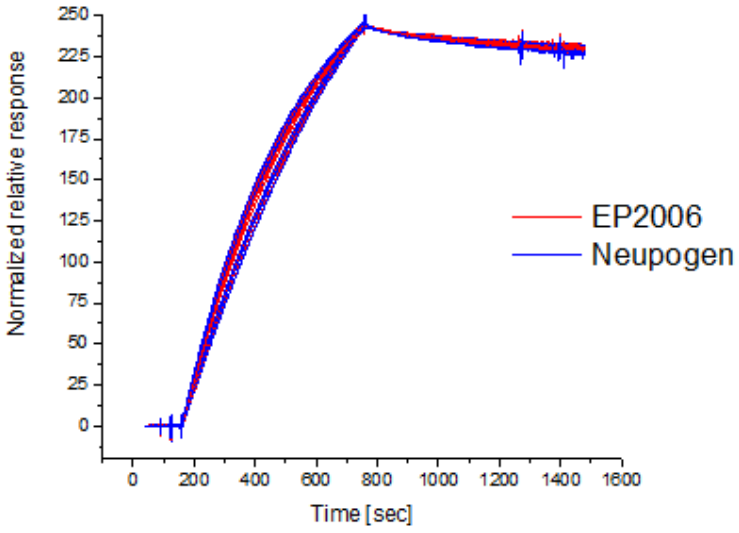
Binding affinities between filgrastim and its receptor reflect the structural integrity of the molecule as well as the correct three dimensional conformation generated by the amino acid residues within the target binding regions. Consequently, binding is directly connected with efficacy as well as with safety because the side effects of filgrastim are a result of its pharmacological activity.

Binding affinity to G-CSFR of both products was tested by surface plasmon resonance (SPR) in a receptor coated flow cell. Calculated k_{on}, k_{off} and KD values were highly similar between ZARXIO and Neupogen (Table 6). Figure 9 shows representative SPR sensograms from the head-to-head biosimilarity study.

Table 6 – Average binding and rate constants of ZARXIO and Neupogen determined by SPR

Product	k_{on} [kM^{-1} s^{-1}]	k_{off} [μs^{-1}]	K_D [pM]
ZARXIO (n=6 lots)	1.1	9.6	87.5
Neupogen (n= 6 lots)	1.2	9.4	80.1

Figure 9 - SPR sensograms from ZARXIO and Neupogen



As can been seen from Table 6 and Figure 9, the binding properties of ZARXIO and Neupogen are highly similar.

7.1.4.2 *In vitro* proliferation bioassay (potency) results demonstrate that ZARXIO and Neupogen are highly similar

The *in vitro* proliferation bioassay is considered a surrogate marker for *in vivo* progenitor proliferation and is therefore representative of the mode of action in the clinical setting. The assay is based on the G-CSF induced proliferation of the murine myelogenous leukemia cell line NFS-60. The potency of the recombinant protein is measured by comparing its proliferative effect with that of a reference preparation and reported in U/mg. Neupogen is defined as a product with a specific activity of $1.0 \pm 0.6 \times 10^8$ U/mg. In Table 7, comparison of minimum-maximum values of ZARXIO and Neupogen are shown. The results show that ZARXIO and Neupogen are highly similar with regards to potency, as measured by the *in vitro* proliferation assay.

Table 7 - Bioactivity: Comparison of min – max values

	Zarxio	Neupogen	Neupogen Prescribing Information
Specific activity in U/mg x 10^8	1.0 – 1.1	0.9 – 1.2	0.4 – 1.6

As can been seen from Table 7, ZARXIO and Neupogen exhibit highly comparable potency.

7.1.5 Size variants

7.1.5.1 Multiple methods establish that ZARXIO and Neupogen have the same size

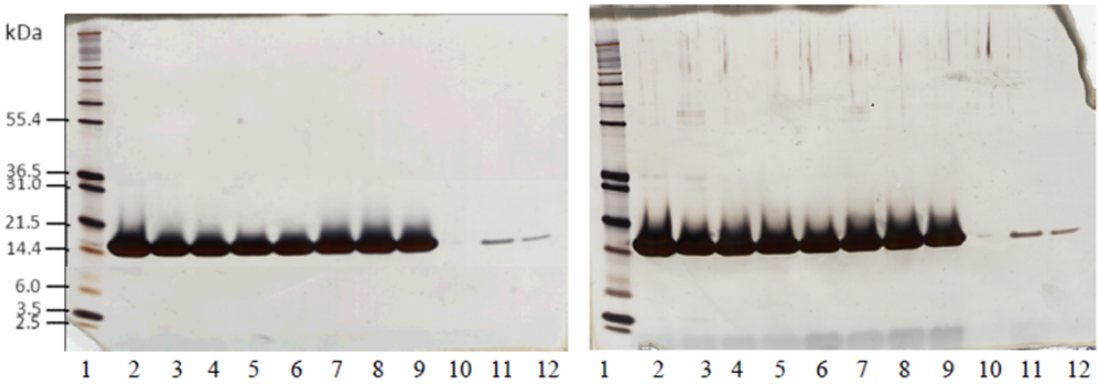
The overall size of a protein and its size variants can be assessed by use of multiple complementary techniques, including gel electrophoresis and size exclusion chromatography.

Examples of size variants are dimers, oligomers, aggregates and lower molecular weight fragments, including N-terminal truncations generated during synthesis in the cell. Aggregates are known to have caused immunogenicity in other products (though not filgrastim) and are therefore tightly controlled in protein-based therapeutics.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a gel-based technique that measures the size of the protein on the based on movement through a gel pores following application of a directed electrical field. Figure 10 shows a representative silver-stained SDS-PAGE gel from the head-to-head biosimilarity exercise. The highly similar migration under both reducing and non-reducing conditions confirms the size similarity and levels of size variants in ZARXIO and Neupogen.

Figure 10 – SDS PAGE of ZARXIO and Neupogen (silver staining)

a. Non-reducing conditions b. Reducing conditions



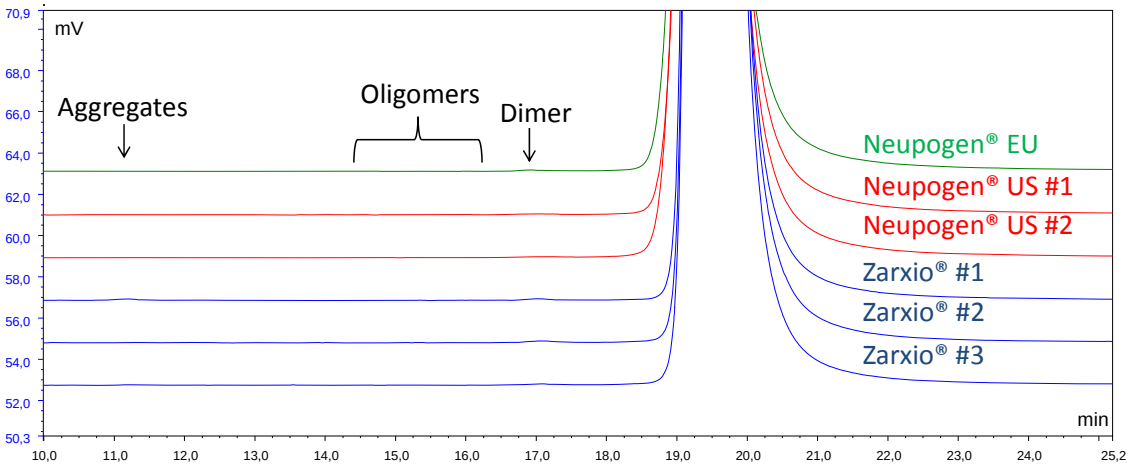
Lane 1: molecular weight markers; Lanes 2, 3, 6, 9: ZARXIO; Lanes 11, 12: Calibration standard; Lanes 4, 5, 7, 8: Neupogen

7.1.5.2 Protein aggregate levels of ZARXIO and Neupogen are highly similar

Size exclusion chromatography is a powerful technique for detection of multimer formation of proteins. All ZARXIO and Neupogen batches evaluated using size exclusion chromatography (SEC) showed a maximum value for High Molecular Weight Variants (HMWV – including dimers, oligomers, and aggregates of ≤0.1 (limit of quantitation of the analytical method) and are therefore highly similar (

Figure 11).

Figure 11 – Size exclusion chromatograms of ZARXIO and Neupogen batches



Size exclusion chromatograms (SEC) of three ZARXIO and three Neupogen batches. The peak at 17 min in the ZARXIO reference standard (EP2006.15REF) is intended and used for annotation of the non-covalent dimer variant.

Proteinaceous particles are a result of continued aggregate formation with diameters of above ~0.1µm. Measurements of proteinaceous subvisible particles by micro flow imaging (MFI) demonstrated a lower number of subvisible particles in ZARXIO as compared to Neupogen.

In summary, size variants, and especially aggregates, are present at very low levels, if it all, in both products, ZARXIO and Neupogen.

7.1.5.3 Oxidized variants are present at low levels

Recombinantly-derived filgrastim has four methionine residues (at position 1, 122, 127 and 138; in contrast to G-CSF which lacks the methionine at position 1) and all four methionines can oxidize to their sulfoxide derivatives (Figure 12). All such variants were found to be at very low levels in all batches analyzed and are highly similar between ZARXIO and Neupogen.

As measured in the *in vitro* proliferation assay, all oxidized minor variants, except the methionine 1 oxidized variant show a decreased potency. This can be explained by the vicinity of methionine 122, 127 and 138 to one of the binding sites in the G-CSF receptor, whereas methionine 1 is located in a flexible (non-functional) region at the protein N-terminus. The known impact on the potency of these three oxidized variants necessitates a thorough comparison between ZARXIO and Neupogen. Oxidized minor variants can be detected and quantified with high sensitivity by RP-HPLC – the most important analytical assay for detecting minor product variants in filgrastim. The upper panel in Figure 12 shows a RP-HPLC chromatogram in full scale demonstrating the high purity of the product, and the lower panel shows a zoom of the baseline region and the assignment of the minor product variants.

Figure 12 – Assignment of variants in RP-HPLC

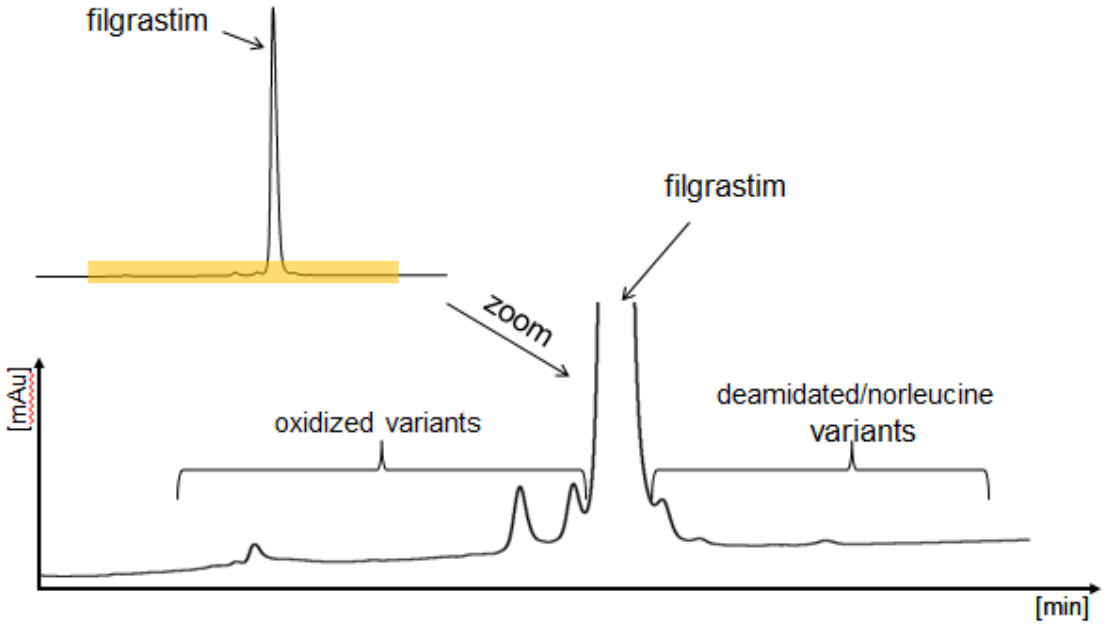
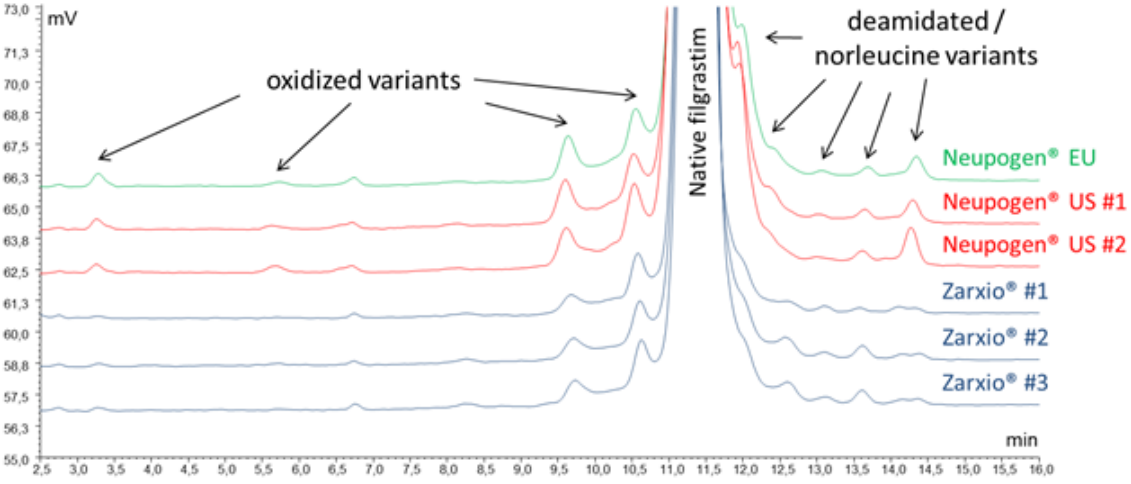


Figure 13 shows the overlays of the RP-HPLC chromatograms of the head-to-head biosimilarity comparisons. The ZARXIO batches V201102, V201001 and V200001 show slightly lower oxidized variants (denoted as VP in Figure 13) as compared to the Neupogen batches 1026606, 1025269 and 1014928.

Figure 13 – Overlay of RP-HPLC chromatogram from the head-to-head biosimilarity exercise



7.1.5.4 Deamidated variants

As summarized in Table 5, our analyses showed that deamidated variants have very low impact on potency, PK/PD and immunogenicity.

Their abundance is, however, dependent on the age of the product. Therefore it was important to monitor deamidated variants and to demonstrate highly similar degradation kinetics between ZARXIO and Neupogen in stability studies. Similar degradation kinetics and the development of deamidated variants with both ZARXIO and Neupogen were observed (data not shown).

Deamidated variants can be assayed by three analytical techniques: RP-HPLC (peaks NP0, NP1, NP3 and NP4 in Figure 13), cation exchange chromatography (CEX) and isoelectric focusing (IEF). Our cumulative analysis of the deamidated peaks shows that, overall, Neupogen contains several percent more of the deamidated variants than ZARXIO when tested in the head-to-head similarity study; CEX and IEF analyses showed the same result. This is explained, in part, by the younger age of the ZARXIO batches used in these studies. When batches of Neupogen and Zarxio that are of comparable age are tested for the presence of deamidated variants, Neupogen contains roughly 1% more deamidated variants, but these low levels of full active variants to be not clinically relevant.

7.1.5.5 Truncated and norleucine variants

N-terminally truncated variants have low, if any, impact on potency, PK/PD and immunogenicity. The presence of these variants was assayed using RP-HPLC-ESI-MS and the measurements demonstrated high similarity between ZARXIO and Neupogen (data not shown).

7.1.6 Strength (total protein content)

ZARXIO is targeted to have the same strength (total content and concentration of G-CSF, as defined by BPCIA) as US-licensed Neupogen. Our analytical results show that ZARXIO strength is in the same range as Neupogen (95% - 103.3% for ZARXIO compared to 96.7% to 105.0% for the declared content of Neupogen) and well within the common accepted industry range of 95%- 105% for declared content.

7.1.7 Stability profiles of ZARXIO and Neupogen are highly similar

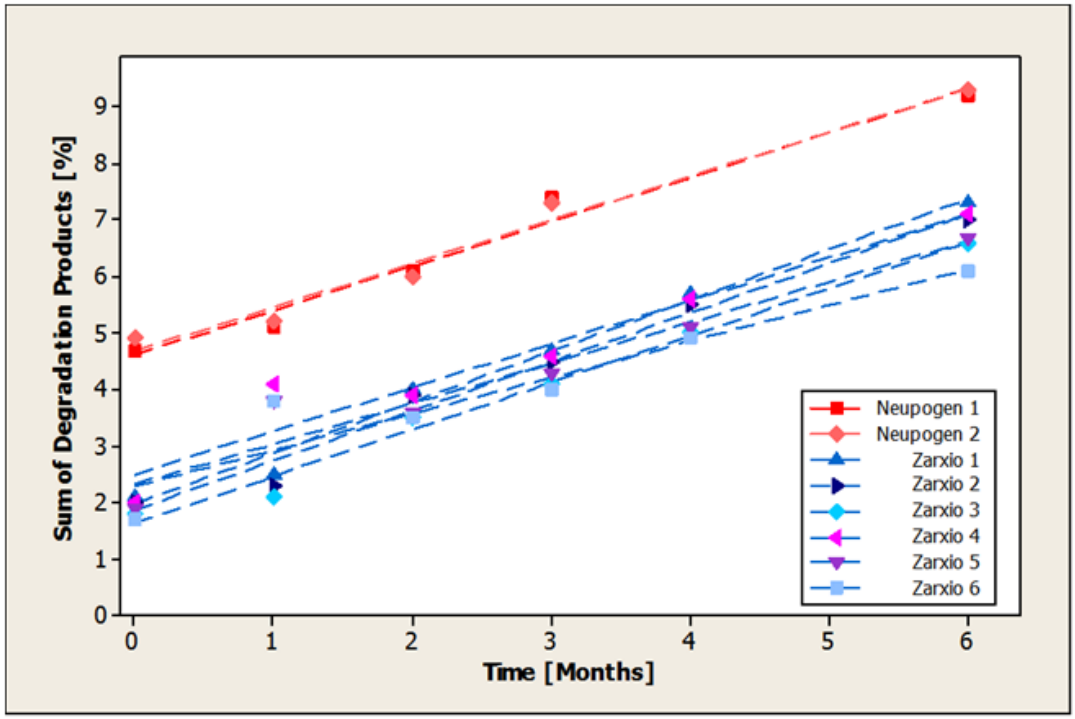
A comprehensive panel of stability indicating assays was set up to assess and compare the stability of ZARXIO and Neupogen under recommended storage conditions as well as at accelerated storage conditions. These included measures of potency, protein concentration and degradation levels over time. Special attention was paid to the clinically relevant product attributes (see Table 5).

No appreciable differences were detected in either the *in vitro* proliferation or the protein concentration assays following storage under recommended temperatures (data not shown).

According to ICH guideline Q5C, accelerated storage conditions are used to reveal potentially hidden molecular differences between products by comparing rates of degradation. Figure 14 shows a comparison of the rates of decay of ZARXIO and Neupogen stored at 25 °C (significantly higher than the recommended storage condition of 5 °C). RP-HPLC analyses

reveal that ZARXIO and Neupogen show similar overall degradation rates of 0.79%/month and 0.78%/month, respectively. The difference in the intercepts seen for ZARXIO and Neupogen arises mostly from the different ages of the products at the start of study (Neupogen was older because it was purchased on the open market). Neupogen was placed on the accelerated stability study 18 months before its expiration date while ZARXIO was placed on the stability study 30 months before expiration.

Figure 14 – Increase of degradation products under accelerated conditions (25 °C)



7.1.8 Conclusion: analytical comparisons on multiple levels and with multiple assays confirm that ZARXIO and Neupogen are highly similar

ZARXIO was developed to be highly similar in all clinically relevant quality attributes to its reference product Neupogen. The comprehensive analytical program that has been conducted confirms that ZARXIO is highly similar to Neupogen with regard to sequence and folding structure as well as product variants that could impact potency and safety. It also remains highly similar during product storage, as demonstrated by comparative stability studies.

A fundamental concept of biology is that structure drives function. ZARXIO binds to its receptor(s) in a highly similar fashion as Neupogen and therefore is expected to elicit cellular responses leading to identical clinical effects in all indications for which Neupogen is approved. The biological similarity of ZARXIO and Neupogen was subsequently confirmed in animal and human clinical studies.

7.2 Nonclinical program overview

The key objective of the nonclinical program was to compare general safety, local tolerability and validate PD similarity of ZARXIO and Neupogen *in vivo* at a level of sensitivity and scrutiny which cannot be achieved in humans. Animals were generally dosed subcutaneously, as this corresponds to the predominant clinical use and is expected to be more sensitive for detecting potential differences as intravenous injection, which would exclude the potential impact of absorption processes. Safety, including relative immunogenicity, was assessed sub-acutely in rats and local tolerance was assessed in rabbits. Due to the high homology of filgrastim and its receptor across all species, including humans, results obtained using both species are considered predictive. The assessment of safety included quantification of organ weights and also full histological assessment of samples from all tissues and organs, including bone marrow. Assessment of local tolerability was done through macroscopic scoring and histological assessment of injection sites. This assessment covered the recommended routes of administration (s.c. and i.v.) as well as unintended routes of exposure. The pharmacological characteristics of ZARXIO and Neupogen were compared in both naïve and neutropenic rats. The mechanism of neutropenia induction and the resulting consequences were identical to human patients, i.e. cyclophosphamide (CPA), a myelosuppressive chemotherapeutic. The characterization of PD similarity extended beyond the clinical dose level and covered the entire dose response.

Taken together, five animal studies were performed (Table 8) to compare pharmacodynamics, toxicity, toxicokinetics, and local tolerance. In the nonclinical program, the two PFS strengths of ZARXIO and Neupogen, as approved in the EU, were tested (i.e. 480 and 300 mcg/0.5 mL), corresponding to concentrations of 960 and 600 mcg/mL. The higher concentration was tested in the toxicity, PD and local tolerance studies (EP06-001 and EP06-006). The lower concentration was tested in the PD study (EP06-004). In all studies, the matching PFS strengths of Neupogen were used. Procedures to prepare the application solutions were thus the same for both products at each dose level. With the exception of the local tolerance testing, in all other studies the final drug product was diluted to allow precise and body weight adjusted dosing.

The repeated dose toxicity study (EP06-006 [pivotal toxicity study]), the local tolerance study (EP06-003) as well as the pharmacology study (EP06-004) were completed with the formulation containing 10 mM glutamate, which is the formulation currently on the market in many countries and intended for market supply in the US (all but one clinical studies were undertaken with the glutamate formulation). Dilutions for study EP06-004 were prepared using glutamate buffer for both products, making it again most sensitive for potential differences resulting from the API. In the pivotal safety study EP06-006, the matching dilution solution, i.e. glutamate buffer, was used to dilute ZARXIO and acetate buffer to dilute Neupogen, which made the study most predictive for the clinical outcome. The supportive toxicity study (EP06-001) and the toxicokinetic (TK) study (EP06-002) were conducted using ZARXIO drug substance that was formulated with a 10 mM acetate buffer (the same buffer as the US-licensed Neupogen). This comparison was most sensitive to detect potential differences resulting from the API because it excluded any impact of the formulation.

Table 8 – Overview of nonclinical development program

Study number	Species	Goal and design of study	Dose level, Injection volume, Route	Group size, Gender	GLP-compliant	TK / PK	PD / Efficacy	Local tolerance	Toxicity	Immunogenicity
EP06-001	Rats/Wistar	Comparison of toxicity upon 4-week treatment, parallel, multiple dose, 6 week recovery	Placebo (acetate buffer), 20, 100, 500 mcg/kg ZARXIO 20, 500 mcg/kg b.w. Neupogen, 2 ml/kg, s.c.	10-15 M&F	X	X	X	-	X	X
EP06-002	Rats/Wistar	Comparison of TK upon 2-week treatment, parallel, multiple dose	20, 100, 500 mcg/kg ZARXIO, 20, 500 mcg/kg Neupogen, 2 ml/kg, s.c.	10 M	X	X	-	-	-	-
EP06-003	Rabbit/New Zealand White	Comparison of local tolerance, single dose	Placebo (0.9% saline), 500 µl ZARXIO, Neupogen and placebo, 500 µl/injection, s.c., intravenous, intramuscular, intraarterial, paravenous	12 F	X	-	-	X	-	-
EP06-004	Rats/CD	Comparison of PD in naive or neutropenic (cyclophosphamide induced) animals, parallel, single dose	Placebo (glutamate buffer), *Naive:* 10, 20, 40, 80, 160 mcg/kg ZARXIO or Neupogen, 0.67 - 2 ml/kg, s.c. *Neutropenic*: 30, 60, 100 mcg/kg ZARXIO or Neupogen, 2 ml/kg, s.c.	12 M	X	-	X	-	-	-
EP06-006	Rats/Wistar	Comparison of toxicity upon 4-week treatment, parallel, multiple dose, 6 week recovery	Placebo (glutamate buffer), 20, 100, 500 mcg/kg ZARXIO 20, 500 mcg/kg Neupogen, 1.3 or 2 ml/kg, s.c.	9-15 M&F	X	X	X	X	X	X

b.w.=body weight; s.c.= subcutaneous; PD=pharmacodynamics; PK=pharmacokinetics; TK=toxicokinetics

Primary Pharmacodynamics

ZARXIO and Neupogen demonstrated similar increases in neutrophil count, in both naïve and neutropenic rats (study EP06-004). In both settings the products were injected subcutaneously, as this corresponds to the typical route of administration in the clinical setting. A broad range of doses were tested and a dense sampling scheme ensured close monitoring of the ANC to allow a reliable assessment of the PD response. Both products were diluted using the buffer system of ZARXIO, an approach which made the comparison most sensitive to potential differences of the API. Furthermore, covering the response curve in a single study ensured a homogenous background, correspondingly low variability and thus high sensitivity for detection of potential differences. The naïve setting was chosen as the fully competent neutrophil precursor cells, i.e. showing the maximal response to the pharmacological stimulation by G-CSF. Accordingly the effect size of G-CSF is the highest in this setting, which makes this the most sensitive setting. On the other hand, it does not fully reflect the clinical use in neutropenia. Accordingly, the comparison of the dose-response characteristic was also performed following treatment with cyclophosphamide, resulting in neutropenia. The mechanism causing neutropenia and the consequences resulting are identical to patients, which provides results from this model with a high predictive validity.

Considering the high responsiveness of the bone marrow in the naïve setting, the neutrophil response to ZARXIO and Neupogen was compared at 10, 20, 40, 80 or 160 mcg/kg. Naïve, nine weeks old CD® rats were treated repetitively, i.e. for four days of administration. Group size was n=12/group, absolute neutrophil count (ANC) was followed until study day 12. The primary endpoint for pharmacodynamics response evaluation was the area under the effect curve (AUEC) for ANC. ZARXIO and Neupogen consistently induced a marked and dose-dependent leukocytosis and neutrophilia, with less pronounced increases in monocytes, eosinophils and basophils. As shown in Figure 15, treatment with ZARXIO (upper panel) or EU-authorized Neupogen (lower panel) resulted in a dose dependent increase of ANC. As detailed below in Table 9, the comparison of the $AUEC_{0\text{-}12days}$ and maximal ANC (E_{max}) by means of 95% confidence intervals (CI) for the ratio of the means for ANC showed that ZARXIO and Neupogen are comparable at all doses tested.

Table 9 – Comparison of ANC in naive rats, including ratio of means (ZARXIO/Neupogen)

AUEC[a] [cells x 10^3 / L x days]

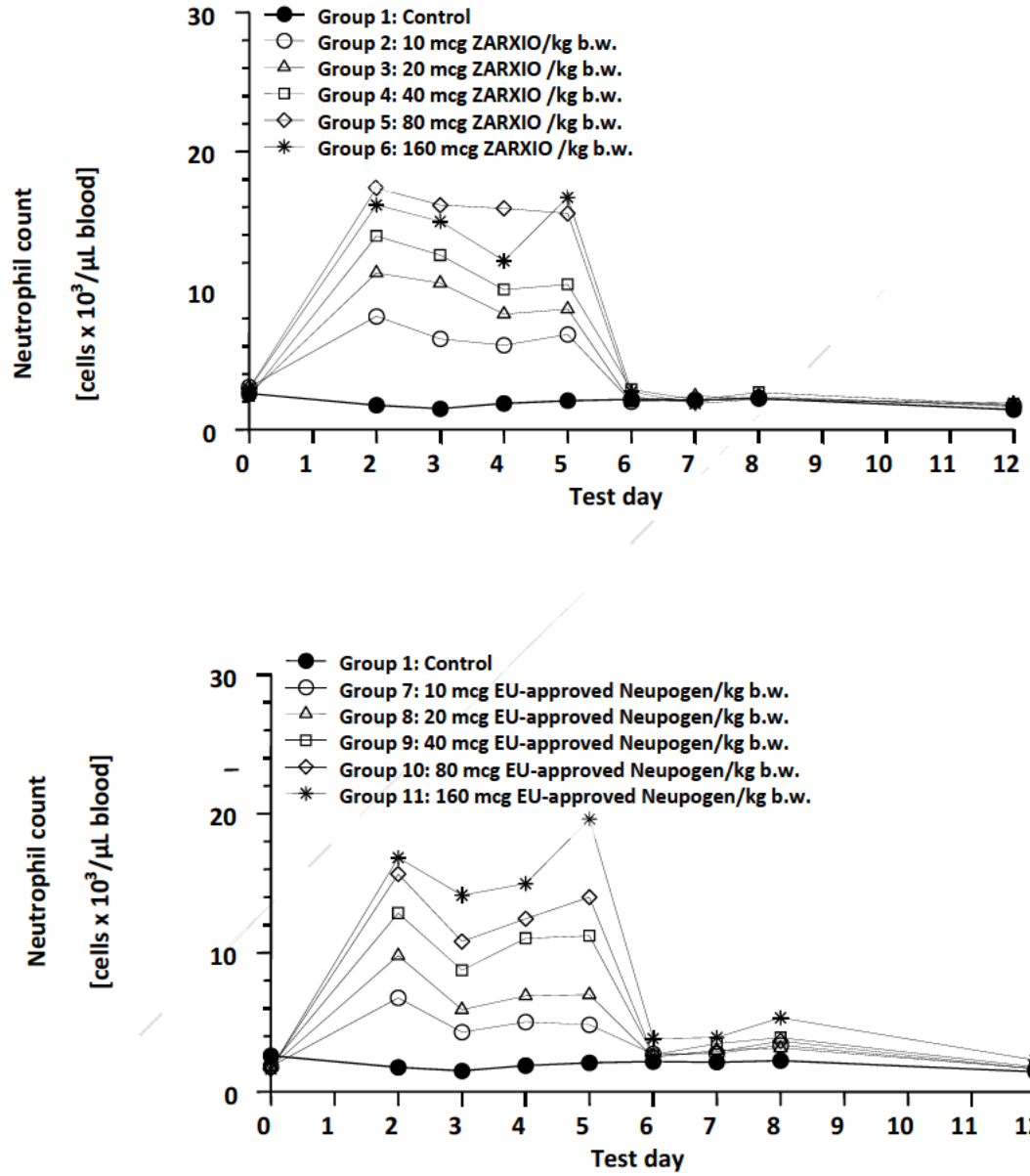
Dose	Means		Median		Ratio means	95% CI
	ZARXIO	Neupogen	ZARXIO	Neupogen		
10 mcg/kg	47.99	43.14	46.2	41.1	1.12	0.95-1.33
20 mcg/kg	60.41	52.86	57.2	52.2	1.15	0.97-1.36
40 mcg/kg	71.60	71.50	70.4	74.3	1.02	0.86-1.21
80 mcg/kg	90.32	80.25	83.3	83.5	1.12	0.95-1.33
160 mcg/kg	84.85	101.15	81.2	98.2	0.85	0.72-1.01

E_{max} [cells x 10^3 / L]

Dose	Means		Median		Ratio means	95% CI
	ZARXIO	Neupogen	ZARXIO	Neupogen		
10 mcg/kg	8.80	6.96	8.5	6.8	1.28	1.08-1.51
20 mcg/kg	11.84	10.07	10.6	10.3	1.17	0.99-1.39
40 mcg/kg	14.29	13.56	14.5	13.0	1.06	0.90-1.25
80 mcg/kg	18.86	16.42	19.2	16.5	1.14	0.96-1.35
160 mcg/kg	18.95	20.27	18.1	22.7	0.94	0.80-1.11

[a] The AUEC in the interval 0 to 12 days for ANC was calculated by trapezoidal integration. Test and comparator product were compared using 95% confidence intervals for the ratio of the means; [b] ratio=test / comparator

Figure 15 - Mean ANCs obtained in naive CD rats following four s.c. treatments with ZARXIO or EU-authorized Neupogen



Similar results were also demonstrated in neutropenic rats. Considering the CPA induced myelosuppression, a less pronounced responsiveness of the bone marrow as compared to the naïve setting was expected, including plateauing of the response at a lower dose level. Accordingly, neutrophil response to ZARXIO and Neupogen was compared 30, 60, and 100 mcg/kg. On day 0, neutropenia was induced by a single intraperitoneal dose of 50 mg/kg CPA. ZARXIO, Neupogen and placebo were administered repetitively, i.e. for four

consecutive days (day 1-4). Group size was n=12/group, absolute neutrophil count (ANC) was followed until study day 12. The animals used were of the same age and strain as those used for the naïve setting.

All active treatment groups showed significantly ($p \leq 0.01$) higher ANC results for both $AUEC_{0\text{-}12d}$ and E_{max} as compared to the control group. The time course for both products is illustrated in

Figure 16. As shown in Table 10, the comparison of the test and comparator product of equal strength by means of 95% CI for the ratio of the means showed similarity between all test and corresponding comparator product. Duration of neutropenia was a second readout, as defined as number of days with ANC < 1.0 x 103 /l. The median duration of neutropenia was 1 week in placebo treated neutropenic group and shortened to 1 day in all groups treated with ZARXIO or Neupogen.

Table 10 - Comparison of ANC in neutropenic rats, including ratio of means (ZARXIO/Neupogen)

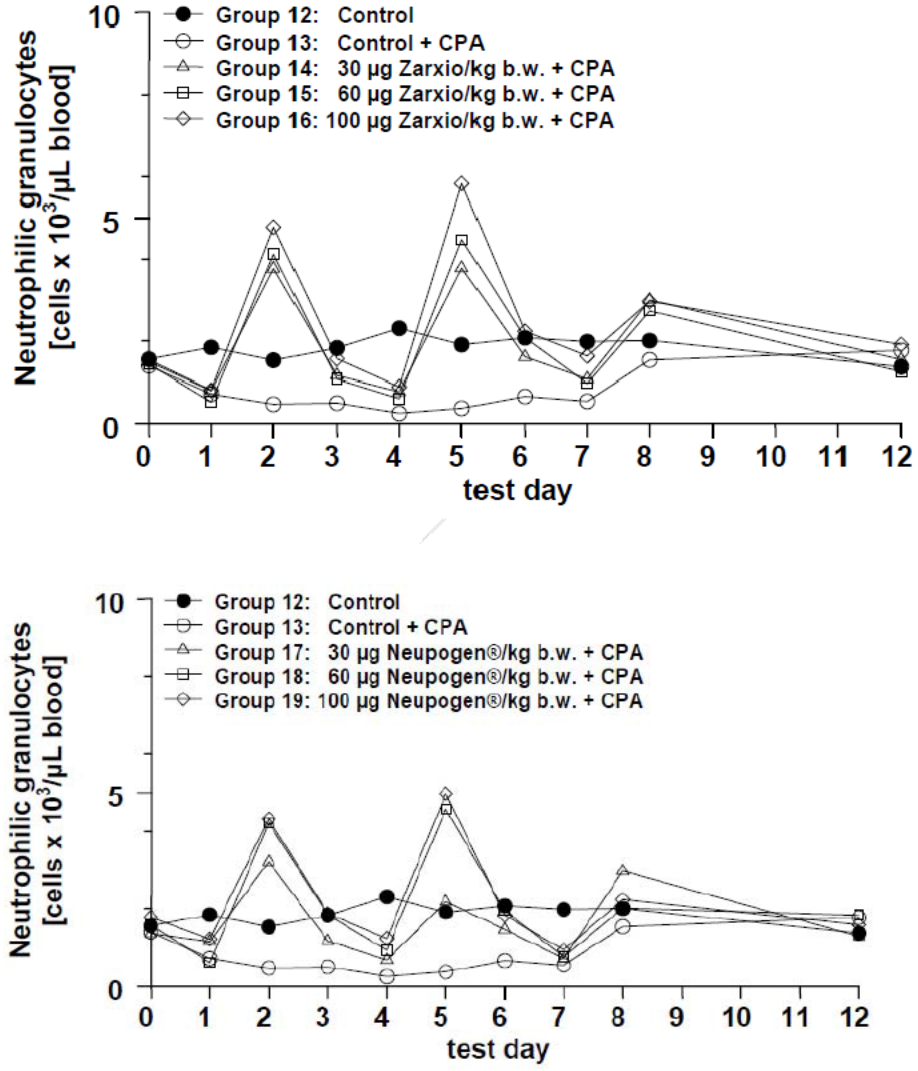
AUEC[a] [cells x 10^3 / L x days]

Dose	Means		Median		Ratio means	95% CI
	ZARXIO	Neupogen	ZARXIO	Neupogen		
30 mcg/kg	24.25	21.20	22.7	21.0	1.10	0.91-1.40
60 mcg/kg	24.34	24.32	26.3	25.4	1.00	0.80-1.25
100 mcg/kg	28.96	24.95	27.0	25.4	1.09	0.87-1.35

E_{max} [cells x 10^3 / L]

Dose	Means		Median		Ratio means	95% CI
	ZARXIO	Neupogen	ZARXIO	Neupogen		
30 mcg/kg	4.95	4.29	2.0	4.6	1.14	0.85-1.51
60 mcg/kg	5.27	5.78	4.3	5.0	0.93	0.69-1.24
100 mcg/kg	6.53	5.78	5.5	5.9	1.12	0.84-1.51

Figure 16 - Mean ANCs obtained in neutropenic CD rats following four s.c. treatments with ZARXIO or EU-authorized Neupogen



Toxicology

Two repeat dose toxicity studies with a recovery period and toxicokinetic assessment were performed in male and female Wistar rats to compare the safety profile of ZARXIO and Neupogen. One study (EP06-006) compared both products diluted in their respective buffer system. This made the comparison most predictive for the human setting. The second study (EP06-001) compared both products diluted in the same Neupogen buffer system. This minimized any potential bias that may result from the formulations. The highest dose level evaluated was greater than the expected human clinical dose by fivefold. The assessment was comprehensive and encompassed quantification of organ weights and also full histological assessment of all tissues and organs, including bone marrow.

In study EP06-006, which is considered the pivotal toxicity study, 9-15 rats per group were treated daily for 28 days with ZARXIO or Neupogen and serum kinetics were evaluated on day 3, 14 and 28 to assess the systemic availability of the two drugs. ZARXIO was tested at doses of 20, 100, or 500 mcg/kg b.w. and Neupogen at doses of 20 and 500 mcg/kg b.w. Both products showed dose-exposure linearity regarding C_{max} and AUC. AUC and C_{max} ratios were close to 1. These findings were supported by the earlier EP06-002 study.

The toxicological findings for EP06-006 were found to be in-line with previously reported animal studies of G-GSF. Table 11, summarizes the relevant toxicological findings from the ZARXIO repeated dose toxicity studies and compares them to the US-licensed Neupogen product information and to the published filgrastim toxicology report by Keller, et al. (Keller 1993). The bone marrow was considered as major target organ, hypercellularity of the bone marrow was found in all dose groups, and well corresponding with the clinical findings of hind limb swelling, osteoporosis and myelofibrosis occurred in few animals. The splenomegaly observed is a known finding caused by filgrastim and considered to represent an exaggerated pharmacodynamic effect. Overall, all findings were considered to be similar between the two drugs.

Table 11 – Overview of main findings from Sandoz-conducted toxicity studies and Neupogen-reported studies

	Neupogen US Prescribing Information [a]	**Keller** [b]	**Sandoz study EP06-006**	**Sandoz study EP06-001**
Unscheduled deaths	No deaths in mice, rats, monkeys at dose levels up to 3450 mcg/kg. Deaths in monkeys with extreme peripheral leukocytosis.	Intracerebral hemorrhage exclusively in simian primates; possibly through increased blood viscosity.	No deaths occurred.	No deaths occurred.
Body weight	Not reported.	Slight reduction in body weight gain in dogs and monkeys.	No treatment-related influence was noted.	Transient reduction in body weight gain of male rats towards the end of the treatment.
Organ weight	Dose-dependent increase in spleen weights in all species.	Increased organ weights.	Dose-dependent increase in spleen weights.	Dose-dependent increase in spleen weights.
Macroscopic changes	Articular swelling of the hind legs in rats, some hind led dysfunction; symptoms reversible after discontinuation of treatment.	Articular swelling of the hind legs in rats; symptoms reversible after discontinuation of treatment.	Slight to marked swollen joints of the hind legs in males with high (500mcg/kg b.w.) doses.	Articular swelling of the hind legs in rats, some hind leg dysfunction; symptoms reversible after discontinuation of treatment.
Histopathology	Osteoclasis and osteoanagenesis in rats; symptoms reversible within 4-5 weeks after discontinuation of treatment.	Osteoclasis and osteoanagenesis in hind legs rats; symptoms reversible after discontinuation of treatment.	Hyperplasia of myeloid cells; increase of hematopoietic cells in bone marrow and spleen; myelofibrosis in the liver.	Riddled compacta or myelofibrosis in the femur bone.
Alkaline phosphates	Dose-dependent increase in rats and monkeys.	Slight to moderate increase in rats.	Dose-dependent increase.	Dose-dependent increase.
Antibody formation	Not reported.	Neutralizing anti-rhG-CSF antibodies detected in dogs and monkeys. Response to rhG-CSF	Improved assay compared with EP06-001: No anti-rhG-CSF antibodies in placebo group, but in all dosing groups receiving ZARXIO or	Anti-rhG-CSF antibodies detected in treatment and control rats, independent of drug, dose or

		slightly lower.	Neupogen from day 14 on. ZARXIO was less immunogenic than Neupogen both in terms of number of rats with detectable antibodies and also in the measured anti-rhG-CSF antibody concentrations. No effect on response.	gender. No effect on response.
Hypersensitivity reactions		None observed.	None observed.	None observed.

[a] – Amgen USA 2014 [b] – Keller 1993

Immunogenicity

FDA's guidance recommends that if differences in manufacturing (e.g. impurities or excipients) between the proposed biosimilar product and the reference product may result in different immunogenicity, measurement of anti-protein antibody responses in animals may be important for assessing patient safety (FDA 2012). Since ZARXIO and Neupogen have different buffering systems; comparative immunogenicity testing was completed. The goal of this assessment, consistent with the similarity standard reviewed earlier, is not to assess immunogenicity per se, but to assess the likelihood for relative differences in immunogenicity.

For study EP06-006 an anti-rhG-CSF antibody assay with improved specificity was developed and used to evaluate the immunogenicity of ZARXIO and Neupogen, as compared to the one used in study EP06-001. Using this assay, none of animals treated with placebo (formulation buffer of ZARXIO) had detectable anti-rhG-CSF antibodies, whereas rats receiving either ZARXIO or Neupogen developed anti-rhG-CSF antibodies, which were detected from day 14 onwards. Across all doses, no gender specific differences were observed.. Comparing matched dose levels, 10 rats (out of 56 rats) which received ZARXIO tested positive for anti-rhG-CSF antibodies and 30 out of 56 rats which received Neupogen tested positive. Importantly, the anti-rhG-CSF antibodies were not neutralizing as the PD effect in all dose groups was not affected and ANC levels increased dose-dependently. The frequent occurrence of anti-rhG-CSF antibodies in this study may result from the fact that the API of both products is a human protein, and thus heterologous in rats. The relevance of the lower immunogenicity rate observed for ZARXIO as compared to Neupogen is not known. Relevant immunogenicity was assessed in all human clinical trials and no differences were seen.

Local Tolerance

A local tolerance test performed in female rabbits showed that the local tolerability of the final ZARXIO formulation was similar to Neupogen. In this study, one group (n=12/group) was treated with 480 mcg/0.5 mL ZARXIO, the second with 480 mcg/0.5 mL Neupogen corresponding to the highest concentration. A matching volume of 0.9% saline was injected contralaterally as a control. Both intended (intravenous and s.c.) as well as unintended, incidental routes of exposure (paravenous, intramuscular and intra-arterial) were assessed. Injection sites were monitored on a daily basis, half of the animals were sacrificed for histological evaluation of the injection sites after 2 days and the remaining animals were sacrificed at the end of the observation period.

ZARXIO was equally well tolerated when compared to Neupogen. Furthermore, ZARXIO showed similar local tolerability as compared to the placebo control, both in terms of macroscopic as well as histological assessment, independent of whether exposure occurred via intended or unintended injection routes. An additional group was treated with the early stage acetate formulation (480 mcg/0.5 mL) of ZARXIO as well as saline 0.9% as control, which was also well tolerated.

In conclusion, the nonclinical animal studies demonstrate the similarity between ZARXIO and Neupogen and provide reassurance that the biosimilarity observed in the analytical testing would result in no meaningful clinical differences between ZARXIO and Neupogen.

7.3 Clinical program overview

The ZARXIO clinical development program was undertaken with the goal of confirming the biosimilarity established by the analytical program and to ensure that no clinically meaningful differences between ZARXIO and Neupogen occur such that the safety and effectiveness of the biosimilar is demonstrated to be comparable to that of the reference drug. This is in contrast to the clinical program for a new drug, where the intent of such studies is to demonstrate a potential benefit versus control in terms of efficacy or safety and confirm the acceptability of the new drug benefit-risk profile.

The clinical development program for ZARXIO is comprised of two comparative pivotal studies specifically performed for the US application with US-licensed Neupogen as the reference product – one PK/PD study (EP06-109) in healthy volunteers and one comparative safety and efficacy study (EP06-302) in breast cancer patients. This data package is complemented by supportive data generated in four PK/PD studies in healthy volunteers, a single-arm safety and efficacy study in breast cancer patients, and data from healthy stem cell donors which were performed as part of the European submission package as well post marketing approval in Europe.

Table **12** provides an overview of the clinical studies conducted including the study population, the dose as well as the objectives of the studies. Studies for which the source of Neupogen is listed as "US" are the pivotal studies for this submission and used US-licensed Neupogen as the reference product, while "EU" refers to studies in which EU-authorized Neupogen was used as the reference product. Analytical data as presented in Section 7.1 show that these two reference products have the same analytical properties and hence, the data generated in the studies using EU-authorized Neupogen are considered supportive for this file.

Table 12 – Overview of human experience in clinical trials

Study Number	Study Population	N	Source Neupogen	Dose	PK	PD	Efficacy	Safety	Immuno-genicity
EP06-109	Healthy volunteers	28	US	10 mcg/kg s.c.	X	X		X	X
EP06-302	Breast cancer patients	218	US	5 mcg/kg s.c.	X		X	X	X
EP06-101	Healthy volunteers	32	EU	10 mcg/kg s.c.	X	X		X	X
EP06-102	Healthy volunteers	24	EU	5 mcg/kg i.v.	X	X		X	X
EP06-103	Healthy volunteers	28 27	EU	2.5 mcg/kg s.c. 5 mcg/kg s.c.	X	X		X	X
EP06-105	Healthy volunteers	23	EU	1 mcg/kg	X	X		X	X
EP06-301	Breast cancer patients	170	-	30 MIU <60kg 48 MIU ≥ 60kg			X	X	X
EP06-501	Healthy stem cell donors	121 of 200	-	10 mcg/kg s.c.			X	X	

The PK/PD studies included a total of 174 healthy volunteers to assess and confirm the biosimilarity of ZARXIO and Neupogen across a wide range of doses and routes of administration. The pharmacodynamic response in terms of the ANC and $CD34^+$ cell counts were evaluated as a surrogate for efficacy in these studies. The ANC directly reflects the change in the number of peripheral neutrophils and the $CD34^+$ cell count is an indicator of peripheral blood progenitor cells (PBPCs) mobilization. Both are well-established clinically relevant markers for the effectiveness of products of the G-CSF class and were the agreed upon endpoints with the FDA. ANC qualifies as a valid marker, as it essentially drives diagnosis (e.g., grade of neutropenia), predicts prognosis (duration of severe neutropenia), and is utilized to monitor G-CSF treatment effects. $CD34^+$ represents a useful marker for the characterization of cells necessary for engraftment of PBPC in recipients after myeloablative

therapy. The bone marrow in healthy subjects, in contrast to myelosuppressed patients, is fully responsive to G-CSF treatment. Therefore, a healthy volunteer study is a very sensitive model for the similarity assessment of the potency of G-CSF treatment for increasing peripheral ANC. Moreover, the effects of G-CSF treatment on stem cell mobilization, assessed by $CD34^+$ cell count, can also be reliably assessed and compared in healthy volunteers. Based on the same mode of action in healthy volunteers and in patients, these data provide a comprehensive confirmation of the biosimilarity in terms of PK profiles as well of the potency of ZARXIO and the reference product.

The safety and efficacy studies were conducted in a total of 388 breast cancer patients with the pivotal study designed to confirm non-inferiority of ZARXIO and US-licensed Neupogen with respect to the duration of severe neutropenia in cycle 1 of a TAC chemotherapy regimen. A non-inferiority design was considered adequate and agreed to by the FDA, based on the high degree of similarity established in the analytical program as well as based on the equivalent PD response established in the animal and healthy volunteer studies.

Sandoz, in agreement with FDA, selected breast cancer as the indication for the pivotal safety and efficacy program for a number of reasons: breast cancer is the most frequent malignant tumor in females (American Cancer Society 2009) and it is the indication in which G-CSF is used most frequently and using TAC chemotherapy provides a very sensitive setting to detect any potential differences based on the established treatment effect of Neupogen in this setting. While Neupogen was initially approved based on establishing superiority compared to Placebo in small cell lung cancer, breast cancer patients with similarly aggressive chemotherapies (e.g., AT) have been used more recently to establish non-inferiority between newer G-CSF class products, such as pegfilgrastim (Neulasta®) (Holmes 2002, Green 2003) and tbo-filgrastim (del Giglio 2008).

Importantly, the pivotal safety and efficacy study provides the final confirmatory evidence that ZARXIO and US-licensed Neupogen are biosimilar. These comparative data are complemented by data for the EU approval wherein efficacy, safety and immunogenicity data were generated in a single-arm safety and efficacy trial in breast cancer patients receiving AT chemotherapy over four cycles as well as the extensive post market experience with the product around the world.

7.3.1 Clinical pharmacology

This section summarizes the pharmacokinetic and pharmacodynamic data as generated in five PK/PD studies in healthy volunteers as well as the PK data collected in a sub-study of the comparative safety and efficacy study in breast cancer patients.

7.3.1.1 PK/PD studies

Five randomized, double-blind, single- and multiple-dose PK/PD studies in a total of 174 healthy volunteers (at doses ranging from 1 to 10 mcg/kg) were conducted to assess PK bioequivalence and PD equivalence of ZARXIO and Neupogen (US-licensed and EU-authorized).

All studies used a common design:

- Single-center
- Randomized
- Double-blind
- Healthy male and female volunteers
- 2-way cross-over design
- Equivalence testing

The dose, route of administration, the number of applications as well as the number of subjects analyzed for PK/PD are summarized in Table 13. The pivotal PK/PD study (EP06-109) had both PK as well as PD (ANC response) as co-primary endpoints, while the other studies either primarily focused on PK or on PD. The distinction between primary (P) and secondary (S) objectives is depicted in the table in the respective columns.

Table 13 – Overview of human experience in clinical trials

Study Number	Origin Neupogen	Dose	i.v. or s.c.	Applications/ Period	N	PK	PD (ANC)	PD ($CD34^+$)
EP06-109	US	10 mcg/kg	s.c.	1	26	P	P	S
EP06-101	EU	10 mcg/kg	s.c.	7	32	P	S	S
EP06-102	EU	5 mcg/kg	i.v.	1	24	P	S	-
EP06-103	EU	2.5 mcg/kg	s.c.	7	28	S	P	S
		5 mcg/kg	s.c.	7	27	S	P	S
EP06-105	EU	1 mcg/kg	s.c.	1	23	S	P	-

Note: P = primary objective; S = secondary objective

All PK/PD studies were conducted using a two-way crossover design with wash-out periods ranging from 10 to 40 days (depending on the study) between treatment periods. By this design, each subject acted as their own control. This design was chosen because it reduces the variability as compared to an inter-subject comparison and therefore provides a more sensitive setting to assess the similarity between the proposed biosimilar and US-licensed or EU-authorized Neupogen, respectively.

7.3.1.1.1 Pivotal PK/PD study – EP06-109

The pivotal randomized, double-blind, two-way crossover PK/PD study (EP06-109) was conducted specifically for this application and demonstrates pharmacokinetic and pharmacodynamic equivalence of ZARXIO and the reference product. This study fulfills the recommendations outlined in Section V of FDA's draft Guidance for Industry *Scientific considerations in demonstrating biosimilarity to a reference product (2012)*, and the study design was discussed with the FDA at the pre-IND Type B meeting (2010) and the Biological Product Development Type 4 meeting (2013). As discussed in the guidance, PK and PD data profiles of protein products often cannot be adequately predicted from functional and/or

animal studies alone, so this pivotal study serves as a fundamental component in support of biosimilarity.

The study was set up to have 90% power to prove equivalence in PK (C_{max}, AUC_{last}) and PD (E_{max}, $AUEC_{last}$ of ANC response) within the pre-defined margins of 80-125% for the ratio of the geometric means. The primary analysis was based on the per-protocol (PP) population which constitutes the conservative population for assessing equivalence. The bioequivalence assessment was based on 90% confidence intervals for the ratio of the geometric means corresponding to an overall significance level of 5% based on the two one-sided test procedure. The PD equivalence assessment was initially based on two-sided 95% confidence intervals implying a stricter overall significance level of 2.5%, but upon request from FDA also the 90% confidence intervals for this comparison were calculated.

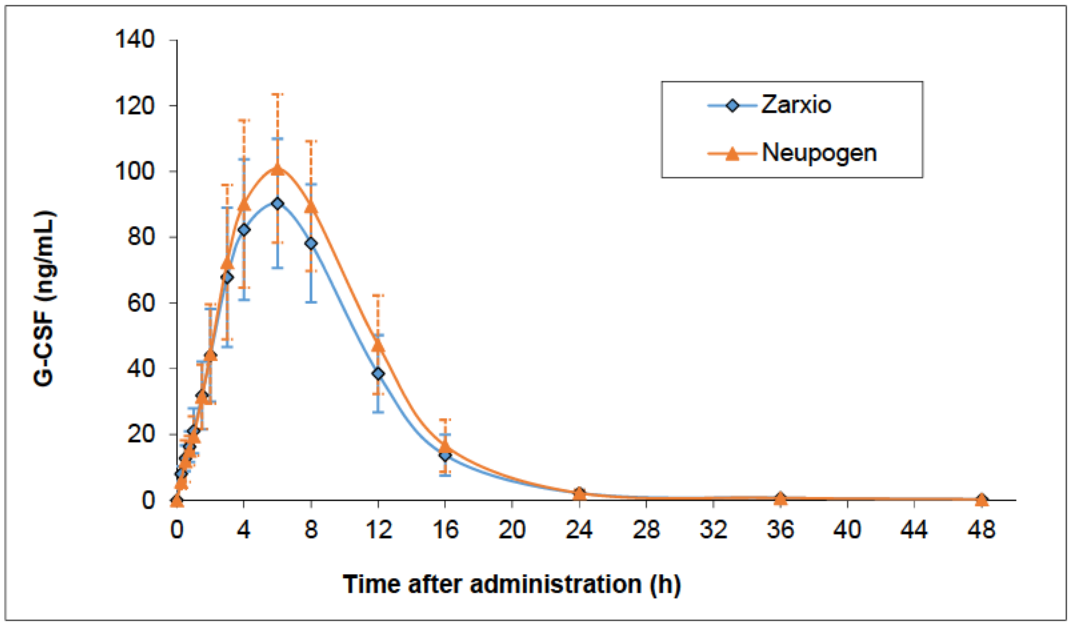
A single-dose of 10 mcg/kg was selected for this study to provide a final confirmation of the biosimilarity of ZARXIO and Neupogen building on the similarity established in prior PK/PD studies with different doses. 10 mcg/kg is the approved dose for the mobilization treatment regimen and is known to elicit a substantial PD response already after one dose for both ANC and $CD34^+$ cells. Being the highest approved dose it also allowed for an adequate comparison of the safety profiles.

The study enrolled 28 adult healthy volunteers and the wash-out time between the single-dose injections in period 1 and period 2 was 28 days. At day one of each treatment period subjects received s.c. 10mcg/kg ZARXIO or Neupogen, both available as a pre-filled syringes, according to the randomized treatment sequence. During each period, a total of 16 blood samples were taken immediately before start of injection and up to 48 hours post-dose for evaluation of pharmacokinetics, a total of 17 blood samples were taken immediately before start of injection and up to 120 hours post-dose for evaluation of ANC and finally a total of 10 blood samples were taken immediately before start of injection up to 336 hours after injection for evaluation of CD34+ PBPC's. All 26 individuals who completed both treatment periods were included in the per-protocol population.

The geometric mean plots of filgrastim serum concentration versus time are presented in

Figure 17.

Figure 17 – Arithmetic mean (+/- SD) filgrastim serum concentration-time profile (study EP06-109, PP population).



The study demonstrated PK bioequivalence of ZARXIO and Neupogen, since the 90% CIs for the ratios of means for $AUC_{0\rightarrow last}$, $AUC_{0\rightarrow\infty}$ and C_{max} were all within the bioequivalence range of 80 to 125% (see Table 14). Recall that ZARXIO (EP-2006) uses a different buffer system due to patent limitations at the time of development and this likely produces the slightly lower PK exposure. As seen below, this slight difference does not impact pharmacodynamic or clinical performance.

Table 14 – Ratios and 90% confidence intervals for mean AUC and C_{max} (study EP06-109, PP population)

	Ratio (%)	90% CI (%)	Intra-Subject CV (%)
$AUC_{0\rightarrow last}$ [ng×h/mL]	87.65	[84.39; 91.04]	7.56
$AUC_{0\rightarrow\infty}$ [ng×h/mL]	87.74	[84.50; 91.10]	7.50
C_{max} [ng/mL]	88.13	[84.00; 92.46]	9.57

AUC = Area under the serum concentration-time curve between the specified time points; C_{max} = Maximum observed serum concentration; CI = Confidence interval; CV = Coefficient of variation; Ratio = ZARXIO/Neupogen

Both products elicited a nearly identical neutrophil and $CD34^+$ cell response with the CIs of the ratios being well within the predefined equivalence range (see Figure 18 and Figure 19 as well as
Table **15** and Table 16 below).

Figure 18 – Arithmetic mean (+/- SD) ANC – time profile (EP06-109, PP population)

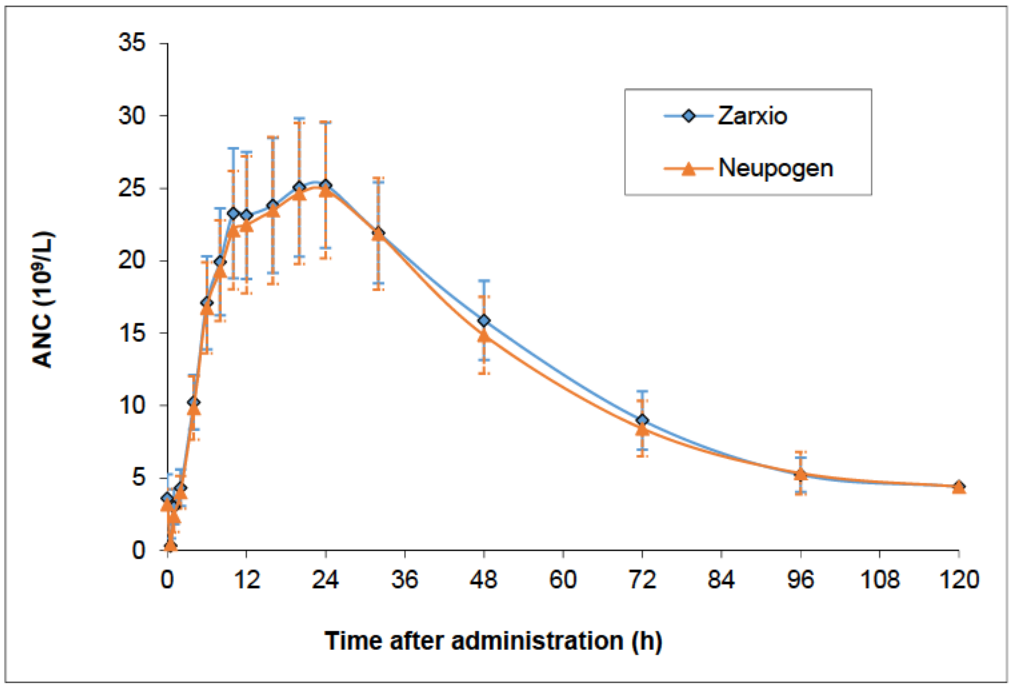


Figure 19 – Arithmetic mean (+/- SD) CD34+ count - time profile (EP06-109, PP population)

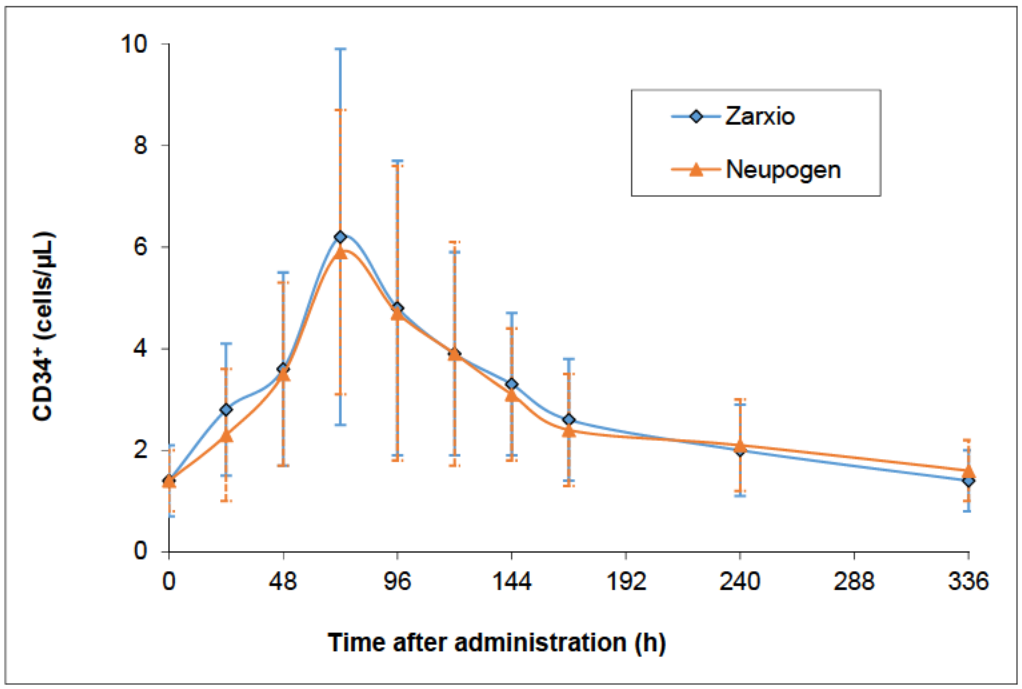


Table 15 – Ratios and 90% and 95% confidence intervals for mean AUEC and E_{max} of the ANC response (study EP06-109, PP population)

	Ratio (%)	90% CI (%)	95% CI (%)
$AUEC_{0\rightarrow120h}$	103.07	[101.45, 105.67]	[100.43; 105.78]
E_{max}	100.33	[97.45, 104.08]	[96.13; 104.70]

AUEC = Area under the response-time curve between the specified time points; E_{max} = Maximum observed PD response; CI = Confidence interval; Ratio = ZARXIO/Neupogen

Table 16 – Ratios and 90% and 95% confidence intervals for mean AUEC and E_{max} of the $CD34^+$ cell response (study EP06-109, PP population)

	Ratio (%)	90% CI (%)	95% CI (%)
$AUEC_{0\rightarrow120h}$	102.29	[95.48, 110.16]	[93.80; 111.55]
E_{max}	104.98	[93.72, 116.86]	[92.11; 119.64]

AUEC = Area under the response-time curve between the specified time points; E_{max} = Maximum observed PD response; CI = Confidence interval; Ratio = ZARXIO/Neupogen

The pivotal PK/PD study confirmed the biosimilarity between ZARXIO and US-licensed Neupogen with respect to PK bioequivalence, equivalence in the ANC response, as well as similar $CD34^+$ cell response.

7.3.1.1.2 Supportive PK/PD studies

The four supportive PK/PD studies which used EU-authorized Neupogen as the reference product were similarly designed and provide a large pool of comparative data for bioequivalence and efficacy assessments across a wide range of doses in single- and multiple-dose settings.

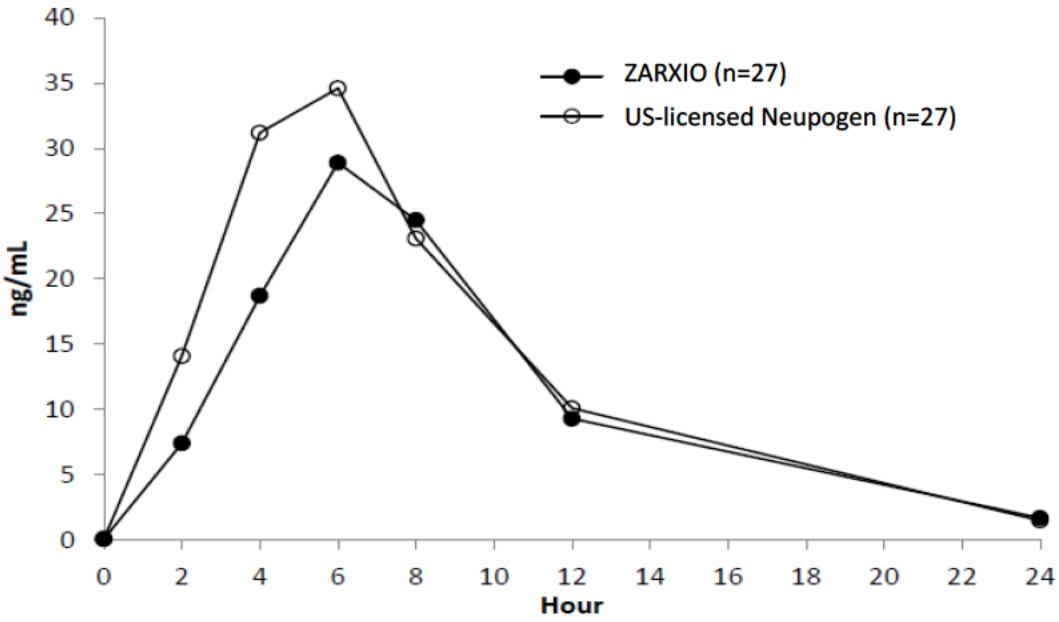
PK bioequivalence could be established in all studies in which this was the primary objective and nearly identical PD responses of both ANC and $CD34^+$ cells were observed in all studies with the corresponding confidence intervals being well within the pre-defined limits. In study EP06-103 which was the pivotal PD study within the European submission, the PD equivalence limits were narrowed to approximately 87-115%. These margins were based on the ANC response data in healthy volunteers for Neupogen (Borleffs 1998) such that at least 85% of the treatment effect of Neupogen will be retained. In general, the PD responses in terms of ANC and $CD34^+$ cells were superimposable across all doses and routes of administration. Both products showed a similar dose-concentration and dose-response behavior and exhibited similarity PK/PD profiles. Detailed results from these studies on the various endpoints can be found in Appendix 1 – PK and PD results from supportive PK/PD studies.

7.3.1.1.3 PK sub-study of pivotal safety and efficacy clinical study – EP06-302

The pivotal safety and efficacy study in breast cancer patients included a PK sub-study to descriptively assess the PK profiles after the first administration as well as the development of the trough levels in cycle 1. 54 patients had a valid PK profile and were included in the PK analysis set.

The mean serum concentration-time curves of both ZARXIO and Neupogen over 24 hours after the first administration of the study drugs are shown in Figure 20.

Figure 20 – Concentration-time curves of ZARXIO and Neupogen after the first dose in Cycle 1 (Day 2) (geometric means) (PK sub-study of pivotal study EP06-302, PK set)



Results from the statistical comparison of $AUC_{0\rightarrow last}$ and C_{max} are shown in Table 17. The mean $AUC_{0\rightarrow last}$ and C_{max} were non-significantly lower for ZARXIO as compared to Neupogen. It should be noted that the sub-study was not powered to assess bioequivalence between the two products, but to descriptively evaluate and compare the PK profiles in a patient setting. Importantly, the apparent initially lower exposure following treatment with ZARXIO did not translate in any pharmacodynamics differences or differences in clinical efficacy.

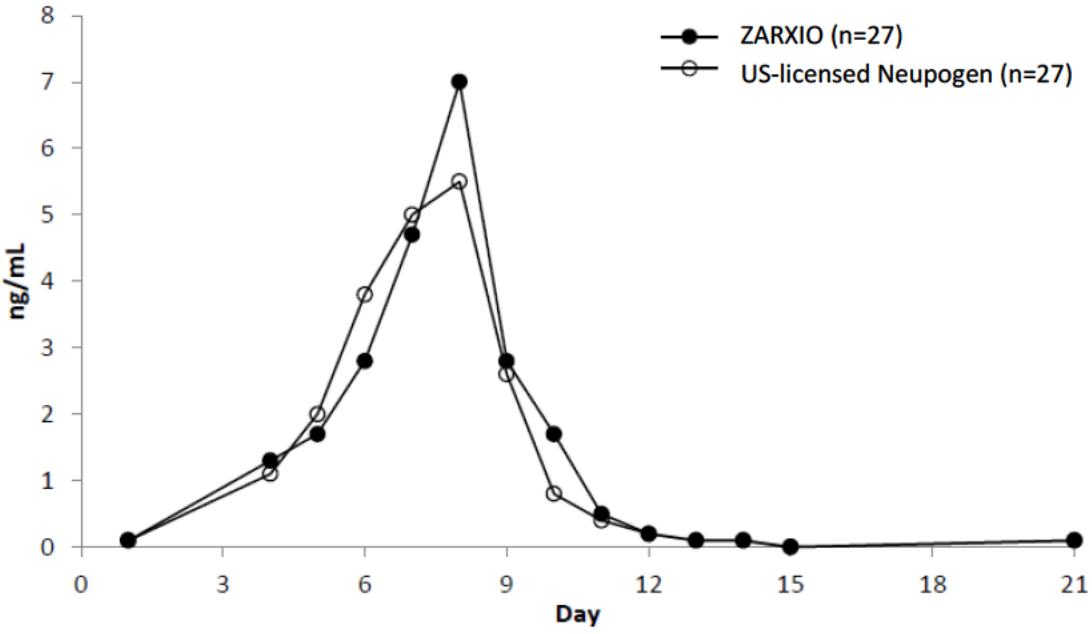
Table 17 – Ratios with 90% confidence intervals for mean AUC and C_{max} (PK substudy of pivotal study EP06-302, PK set)

	Least-square means			
Variable	**ZARXIO**	**Neupogen**	**Ratio**	**90% CI**
$AUC_{0\rightarrow last}$ [ng×h/mL]	269.2	343.9	0.7829	[0.61; 1.00]
C_{max} [ng/mL]	31.2	38.1	0.8193	[0.67; 1.00]

AUC = Area under the serum concentration-time curve between the specified time points; CI = Confidence interval; C_{max} = Maximum observed serum concentration; Least-square mean = geometric mean for log-transformed variables; Ratio = ZARXIO/Neupogen. Note: Calculations were performed using an ANOVA with factor treatment as fixed effect

As presented in Figure 21, trough levels of ZARXIO and Neupogen showed no marked differences: both products had a similar profile without any relevant differences in the accumulation during treatment and with a comparable return to baseline after the last application.

Figure 21 – ZARXIO and Neupogen trough levels in Cycle 1 and on Day 1 of Cycle 2 (Day 21) (geometric means) (PK sub-study of pivotal study EP06-302, PK set)



7.3.1.2 Discussion on apparently lower filgrastim concentrations with ZARXIO

Across all studies a somewhat lower, and in most cases not statistically significant, PK exposure following administrations of ZARXIO was observed. As noted previously, ZARXIO was formulated to contain a different buffer than Neupogen as a result of a patent issue. While Neupogen is formulated in an acetate buffer, ZARXIO has a glutamate buffer. The impact of the formulation was specifically studied in an exploratory PK/PD cross-over study in healthy volunteers (EP06-104) in which the ZARXIO drug substance was formulated in the Neupogen buffer (acetate) and compared to Neupogen as well as to ZARXIO in the to-be marketed formulation (glutamate). In this study, all three products were applied as single-doses of 2.5 mcg/kg. The resulting PK profiles are depicted in Figure 22 with the corresponding results of the statistical comparisons for the PK being shown in

Table 18. When the two drugs are formulated in the same Neupogen buffer system, the PK profiles of are super-imposable and the corresponding 90% confidence intervals clearly demonstrate bioequivalence between the two products.

Figure 22 - Arithmetic mean of serum concentrations of ZARXIO Glutamate, ZARXIO Acetate, and Neupogen (PP population, n = 28)

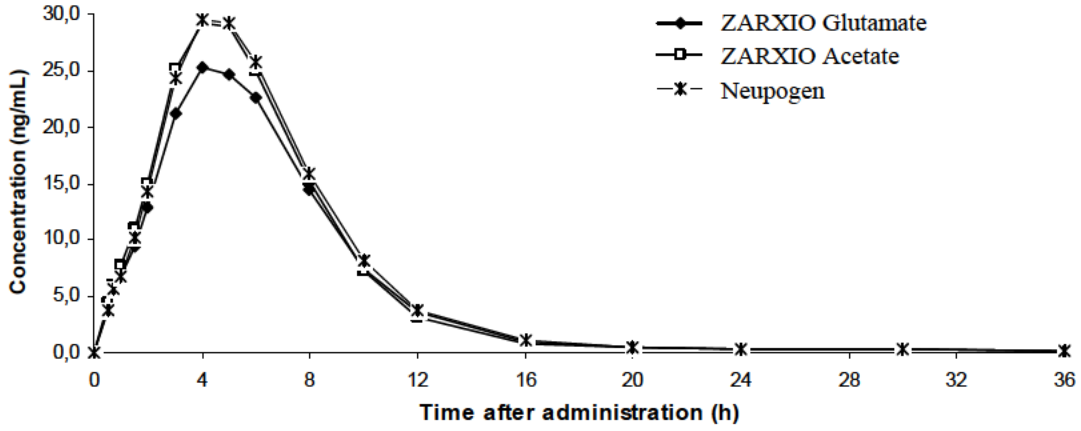


Table 18 – Comparison of the primary pharmacokinetic parameters between the treatments (PP population, n = 28)

Treatment A	Treatment B	Ratio and 90% CI for $AUC_{0\rightarrow last}$ [Treatment A/Treatment B]	Ratio and 90% CI for C_{max} [Treatment A/Treatment B]
ZARXIO Acetate	Neupogen	98.50% [91.69%; 105.81%]	102.16% [93.97%; 111.05%]
ZARXIO Glutamate	EP2006 Acetate	90.77% [84.51%; 97.49%]	82.45% [75.86%; 89.62%]
ZARXIO Glutamate	Neupogen	89.41% [83.23%; 96.05%]	84.23% [77.48%; 91.57%]

As seen in the other PK/PD studies, the differences in PK did not translate into any differences in PD; with nearly identical ANC profiles and confidence intervals for the AUEC and E_{max} ratios being tight around 100% and well within conventional bioequivalence limits and also within the tighter margins (87-115%) established to show PD equivalence. This is not surprising as the relative bioavailability of Neupogen and ZARXIO are highly similar, 61% versus 59% respectively.

The results of this exploratory study show that the difference in the PK exposure is due to the difference in the formulation, but not due to differences in the molecule itself. The relationship between PK exposure and formulations is also further supported by the study EP06-102 in which ZARXIO (glutamate buffer) and Neupogen were administered i.v., established bioequivalence with point estimates close to 100% and very tight confidence intervals (see Table 19 below and also

Figure 23).

Table 19 - Ratios and 90% CIs for the PK parameters following a single i.v. dose of 5 mcg/kg (study EP06-102, PP population, n = 24)

Parameter	Ratio	90% CI
$AUC_{0\text{-last}}$	99.68%	[96.94%; 102.47%]
C_{max}	98.82%	[95.76%; 101.98%]

7.3.2 Clinical pharmacology conclusions

Bioequivalence as well as pharmacodynamic equivalence following single-dose s.c. administrations of 10 mcg/kg ZARXIO and US-licensed Neupogen were established in pivotal study EP06-109. Across all PK/PD studies similar dose-dependent PK and PD profiles could be established. While the filgrastim concentrations appear to be consistently slightly lower for ZARXIO as compared to Neupogen (both US-licensed and EU-authorized), the PD responses for both ANC and $CD34^+$ cells were in all cases superimposable with very tight confidence intervals around 100%. Moreover, the absolute bioavailability of ZARXIO and Neupogen as determined from the i.v. and s.c. single-dose dose profiles in studies EP06-102 and EP06-103, respectively, are nearly identical with values of 0.59 for ZARXIO and 0.61 for Neupogen.

Figure 23 and Figure 24 show the PK-profiles across all single-dose and all multiple-dose studies, respectively.

Figure 23 - Single-dose PK profiles in healthy volunteers following single s.c. and i.v. administrations (studies EP06-102, EP-105, and EP-109)

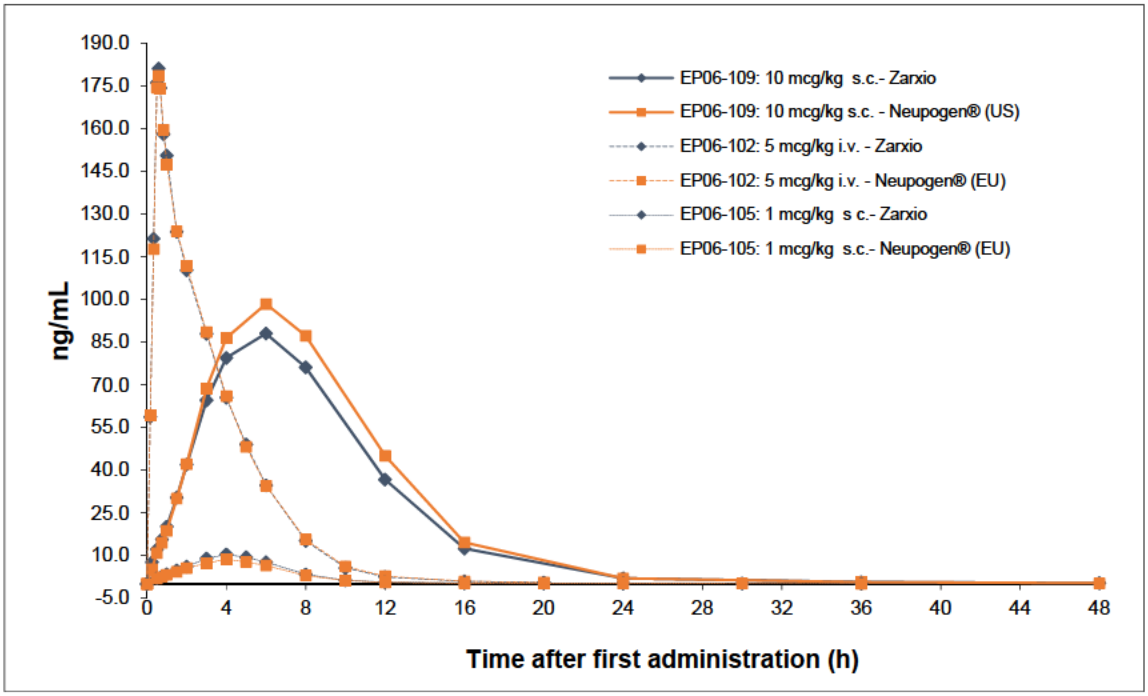
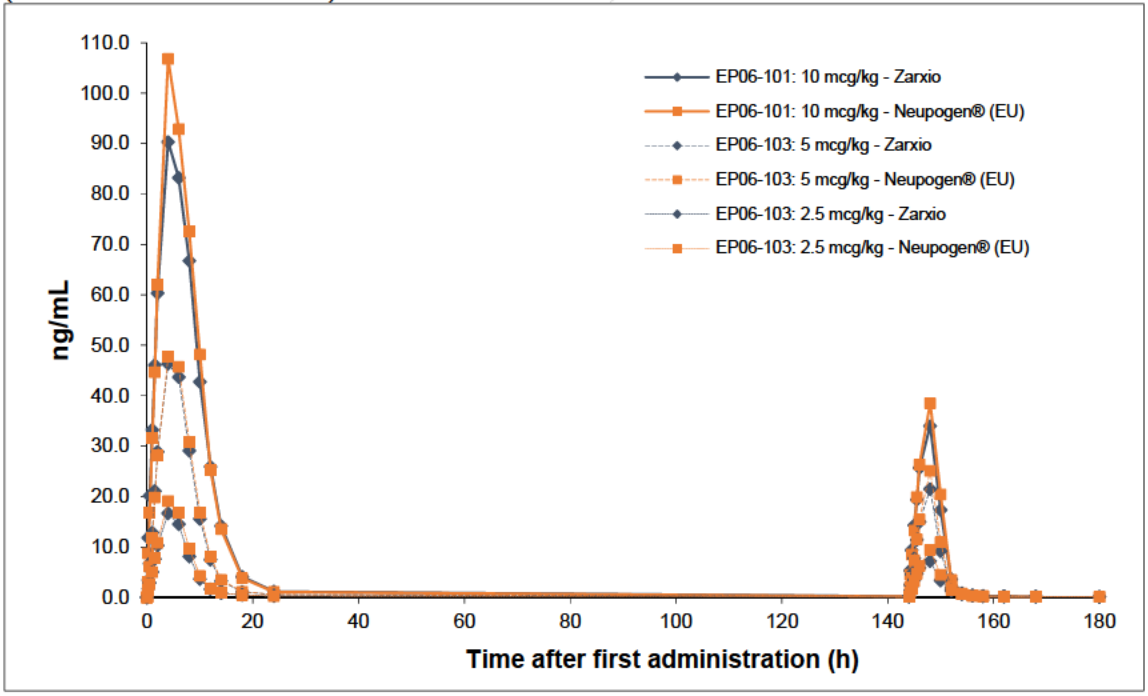


Figure 24 - Single-dose and steady-state s.c. PK profiles in healthy volunteers in multiple-dose studies (studies EP06-101 and EP-103)



The corresponding PD profiles are depicted in Figure 25 to Figure 27 showing superimposable responses in all scenarios tested.

Figure 25 - Dose-response of ANC in healthy volunteers in single-dose studies (EP06-102, EP06-105, EP06-109)

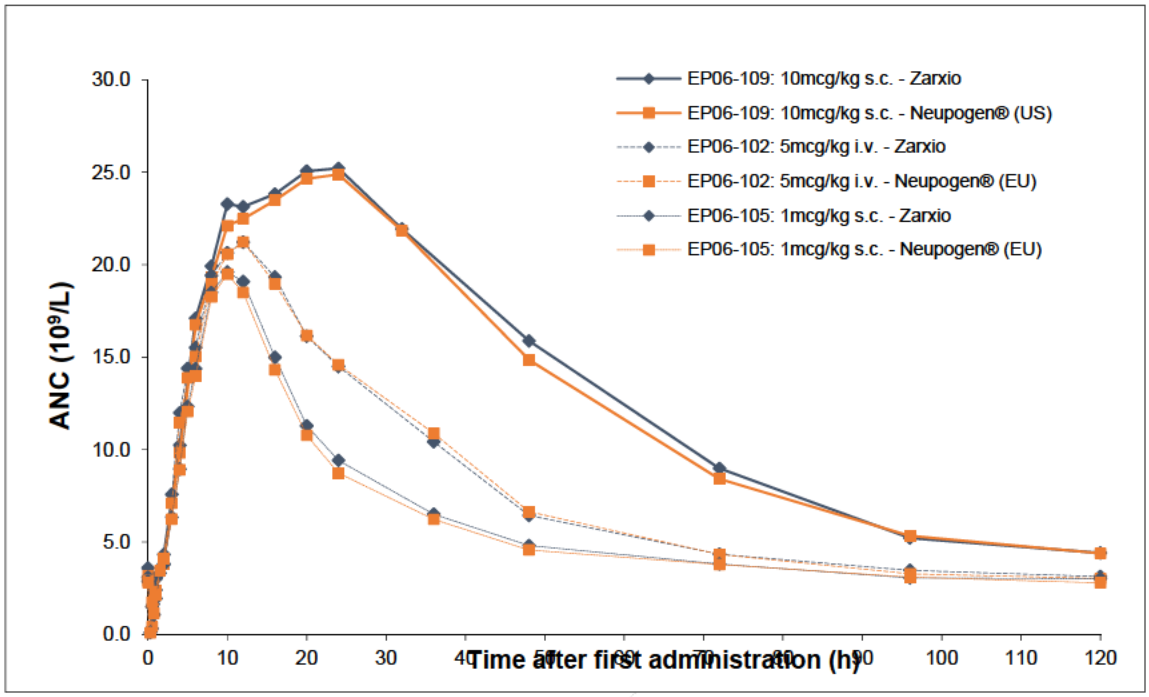


Figure 26 - Dose-response of ANC in multiple-dose studies (EP06-101 and EP06-103)

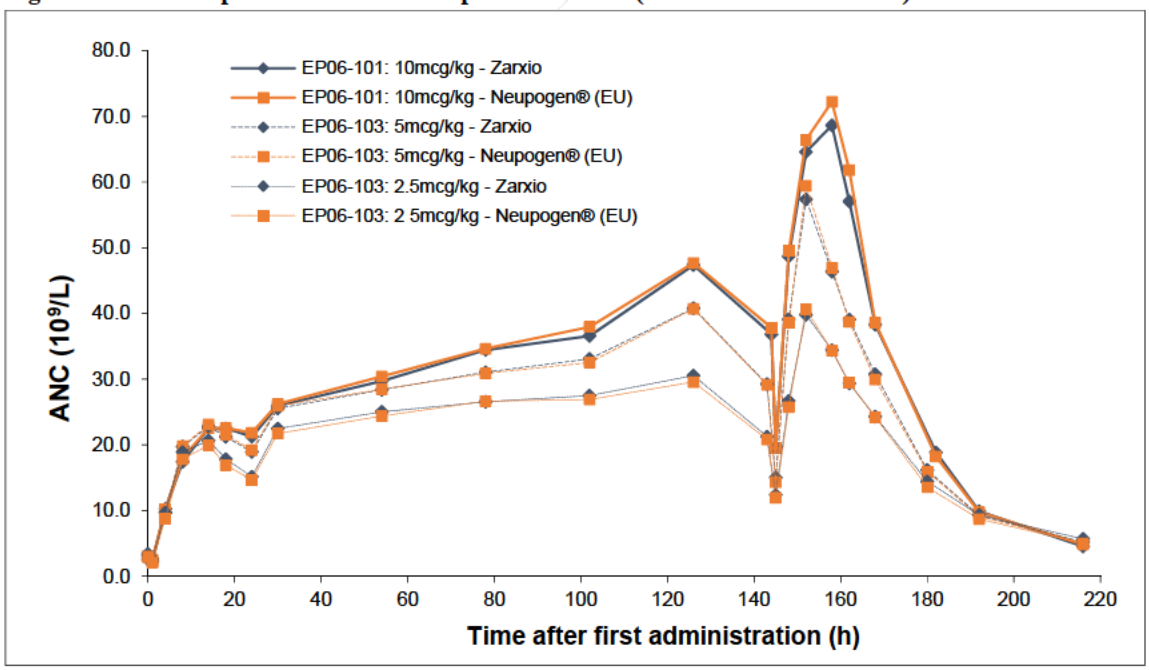
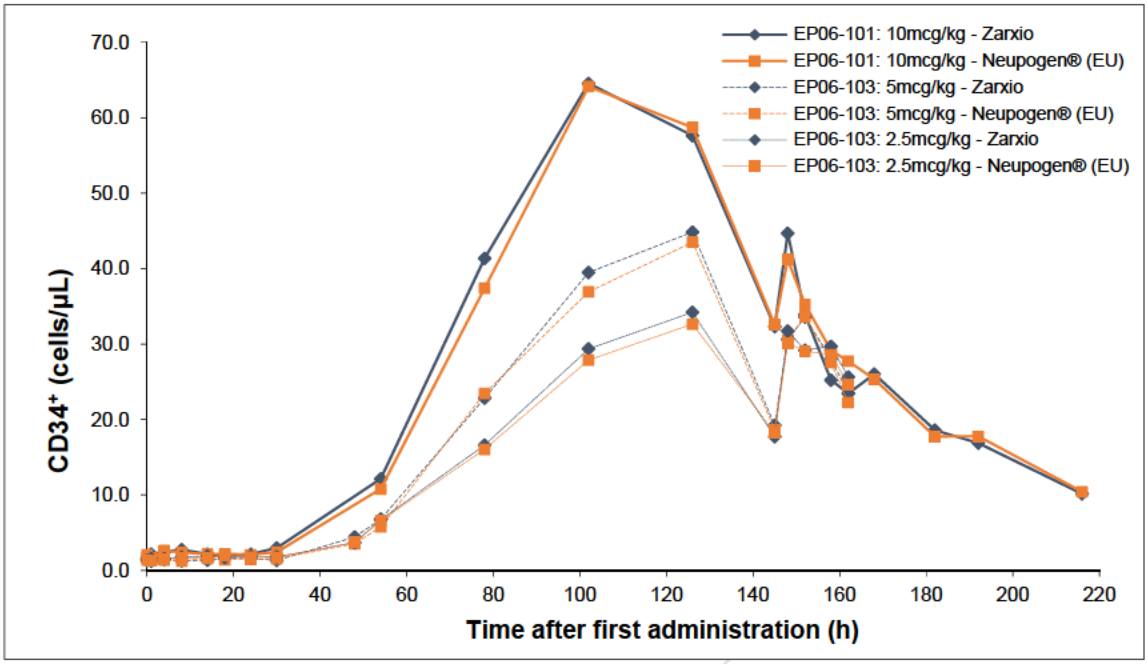
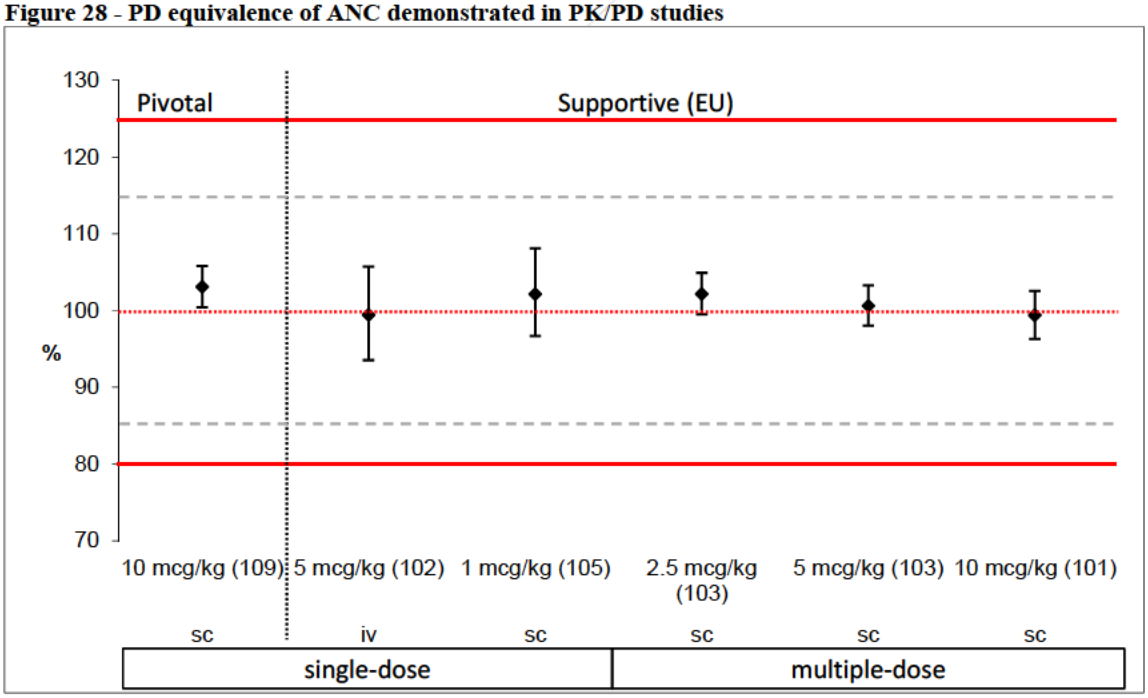


Figure 27 - Dose-response of CD34+ cell counts in multiple-dose studies (EP06-101 and EP06-103)



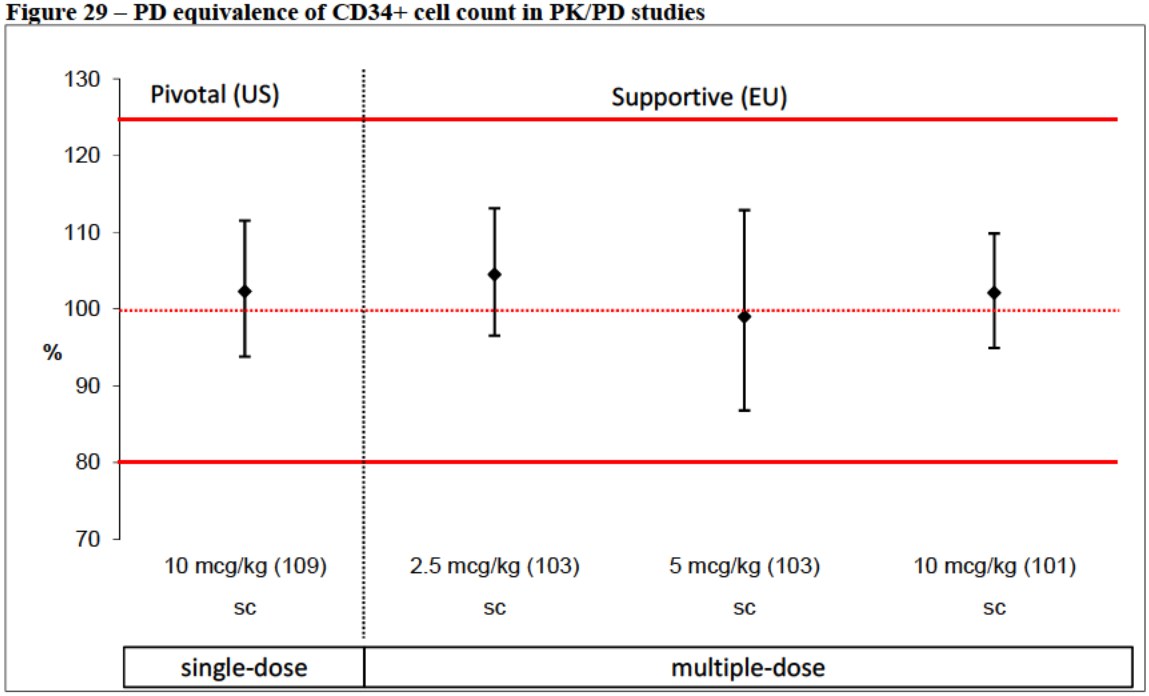
As a consequence, all 95% confidence intervals for the ratios of the geometric mean for the PD parameters were well within the (predefined) equivalence margins.

Figure 28 - PD equivalence of ANC demonstrated in PK/PD studies



Solid lines: standard bioequivalence range 80-125%; dashed lines: narrowed PD equivalence range 87-115%

Figure 29 – PD equivalence of CD34+ cell count in PK/PD studies



Solid lines: standard bioequivalence range 80-125%

In summary, PK bioequivalence and equivalence in ANC response of ZARXIO and US-licensed Neupogen was proven in the pivotal PK/PD study in 28 healthy volunteers. Supportive PK/PD data studies in a total of 134 healthy volunteers covering a dose range from 1 mcg/kg to 10 mcg/kg using both single-dose and multiple-dose application via the s.c. or the iv route, confirmed the highly similar PD response for both ANC and $CD34^+$ cells along with similar PK profiles. Both products show a similar absolute bioavailability and the slightly lower filgrastim concentrations following the administration of ZARXIO are due to a difference in the formulation, but the lower exposure did not lead to any clinically meaningful differences in any of the PD parameters across all doses and for all parameters investigated nor does it have an impact on safety and immunogenicity as further discussed in Sections 8.2 and 8.4.

7.3.3 Clinical studies comparing efficacy and safety of ZARXIO and Neupogen

Two completed safety and efficacy studies (one comparative pivotal study for the US file and one non-comparative study conducted for the EU approval) and an ongoing non-comparative post-authorization safety study provide data to further support a determination of effectiveness. The following table outlines these three studies.

Table 20 – Summary of ZARXIO Clinical Efficacy Studies

Study Number	Design	Study population	Dose and regimen	Objectives	Main efficacy conclusions
Pivotal					
EP06-302	Randomized, double-blind, multi-center safety and efficacy study	218 breast cancer patients receiving six cycles of TAC chemotherapy	ZARXIO and US-licensed Neupogen (supplied in vials, 480 mcg in 1.6 mL), s.c., daily dose of 5 mcg/kg, from Day 2 of each chemotherapy cycle until the ANC recovered to 10×10^9/L after the nadir or up to a maximum of 14 days (whichever occurred first)	Primary: Non-inferiority in clinical effectiveness in terms of duration of severe neutropenia (DSN) Secondary: Safety PK sub-study	ZARXIO was non-inferior to US- licensed Neupogen in DSN in Cycle 1. Mean DSN was 1.17 days for ZARXIO and 1.20 days for Neupogen. The mean difference of DSN between ZARXIO and Neupogen was 0.04 days with a lower limit of the 97.5% CI of -0.26 days, which was well above the non-inferiority margin of -1 day (PP set).
Supportive uncontrolled studies					
EP06-301	Open-label, single-arm, multi-center safety and efficacy study	170 breast cancer patients receiving four cycles of AT chemotherapy	ZARXIO (pre-filled syringes), s.c., daily dose of 5 mcg/kg, from Day 2 of each chemotherapy cycle until the ANC recovered to 10×10^9/L after the nadir or up to a maximum of 14 days	Primary: Safety, tolerability Secondary: Efficacy as prophylaxis of neutropenia	Severe neutropenia was reported in 47% of patients in chemotherapy Cycle 1. The median duration of severe neutropenia was two days. Febrile neutropenia was experienced by 7.6% patients during treatment Cycle 1. Results were in line with what could be expected from literature data.
EP06-501	Open-label, single-arm, post-authorization safety study	121 of targeted 200 healthy unrelated donors evaluated for the interim analysis	ZARXIO (pre-filled syringes) s.c., maximum dose of 10 mcg/kg/day (according to EU label)	Primary: Safety Secondary: Effectiveness of PBPC mobilization	Peripheral blood progenitor cell mobilization in terms of increase in CD34+ cell count was effective in all healthy donors.

7.3.3.1 Pivotal clinical comparison of efficacy study in breast cancer patients for the US file– EP06-302

7.3.3.1.1 Study Design

The clinical efficacy study EP06-302, was a randomized, double-blind, parallel-group, multi-center study conducted specifically for the US BLA application. The study was designed to demonstrate the non-inferiority of ZARXIO to Neupogen in the prevention of neutropenic complications in breast cancer patients treated with established myelosuppressive chemotherapy.

As laid out before, breast cancer was selected as the relevant indication based on its prevalence, the common use of G-CSF in this indication and the fact that it has become a well-established indication for pivotal trials for products in the G-CSF class. TAC was selected as the chemotherapy, since anthracyclines and taxanes constitute an effective group of cytotoxic agents and are part of many breast cancer treatment regimens recommended in evidence-based guidelines. At the time of study conduct (first patient enrolled in 2011) an applied regimen containing anthracyclines and taxanes was the three-drug combination of Taxotere® (docetaxel), Adriamycin® (doxorubicin) and Cytoxan® (cyclophosphamide) (TAC). This TAC regimen consists of docetaxel 75 mg/m^2, doxorubicin 50 mg/m^2, and cyclophosphamide 500 mg/m^2.

According to the breast cancer guidelines at place at the time of study conduct, e.g. by the National Comprehensive Cancer Network, the TAC regimen was recommended as an adjuvant chemotherapy treatment (National Comprehensive Cancer Network 2010a), and it is approved for the adjuvant treatment of breast cancer patients in the US (Taxotere® US Prescribing Information 2008). The TAC regimen is known to lead to significant hematological toxicity and induces febrile neutropenia with a rate > 20% (Smith 2006). With adequate primary neutrophil support from G-CSF products such as filgrastim, this schedule is manageable in the majority of otherwise-fit cancer patients. The use of primary G-CSF prophylaxis with the TAC regimen was recommended by guidelines at the time of study conduct, e.g., by the American Society of Clinical Oncology guideline on the use of hematopoietic colony-stimulating factors (Smith 2006) as well as by the guideline on myeloid growth factors of the National Comprehensive Cancer Network (National Comprehensive Cancer Network 2010b).

The key inclusion and exclusion criteria of the study are listed below:

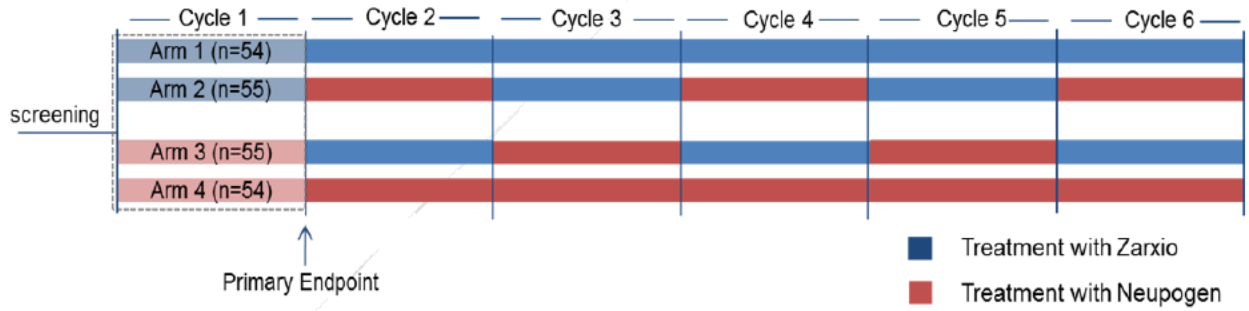
- Key inclusion criteria
  - Patients with histologically proven breast cancer, eligible for neoadjuvant or adjuvant TAC chemotherapy
  - Women ≥ 18 years of age
  - Estimated life expectancy of more than six months
  - Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2
  - Adequate bone marrow function prior to chemotherapy administration
- Key exclusion criteria
  - History of myelogenous leukemia or myelodysplastic syndrome
  - History or presence of sickle cell disease
  - Concurrent or prior radiotherapy within four weeks of randomization
  - Concurrent or prior chemotherapy for breast cancer

- Concurrent or prior anti-cancer treatment for breast cancer such as endocrine therapy, immunotherapy, monoclonal antibodies, and/or biological therapy
- Concurrent prophylactic antibiotics
- Previous therapy with any rhG-CSF product

Study subjects received ZARXIO or US-licensed Neupogen (supplied in vials, 480 mcg/1.6 mL), administered s.c. with a daily dose of 5 mcg/kg starting on Day 2 of each chemotherapy cycle (at least 24 hours after chemotherapy ends) and continued until the ANC recovered to 10×10^9/L after the nadir or up to a maximum of 14 days (whichever occurred first). The total study duration for the individual patient was up to 24 weeks, including up to three weeks screening, approximately 18 weeks of active treatment, i.e. the six TAC chemotherapy cycles with duration of three weeks each, and a follow-up visit about six weeks after the start of the last cycle (approximately four weeks after the last study drug administration).

The treatment sequences are summarized below in Figure 30. The patients were randomized to either ZARXIO or Neupogen in four groups: half of the patients remained on their initial treatment throughout the study (group ZAR and group NEU), and the other half of the patients (groups ZARNEU and NEUZAR) received alternating treatment with ZARXIO or Neupogen starting with the second cycle. This design was implemented to assess the effects of repeated switching for subsequent filing for interchangeability which is not part of this submission.

Figure 30 – Treatment Sequences in Study EP06-302



Arm 1: Patients who received ZARXIO continuously over all six cycles
Arm 2: Patients who received ZARXIO in Cycle 1 and alternating treatment in cycles 2 to 6
Arm 3: Patients who received Neupogen in Cycle 1 and alternating treatment in cycles 2 to 6
Arm 4: Patients who received Neupogen continuously over all six cycles
One cycle consist of 21 days

7.3.3.2 Study objectives and endpoints

The primary objective of EP06-302 was to assess the similar efficacy of ZARXIO compared to Neupogen with respect to the mean duration of severe neutropenia (DSN) during Cycle 1 chemotherapy (mean DSN, the primary endpoint, was defined as the number of consecutive days with Grade 4 neutropenia). DSN is a more sensitive endpoint than the clinical endpoint of febrile neutropenia and it has been shown that the two are correlated. As published by Blackwell and Crawford (Blackwell 1994), the risk of infection, as demonstrated by fever and neutropenia (febrile neutropenia), is related to the severity and duration of neutropenia. Other publications

(e.g. Meza 2003, Bodey 1966) have also shown that a longer duration of severe neutropenia is directly related to an increased risk of febrile neutropenia (FN). DSN can be monitored closely and measured very accurately by daily ANC measurement as implemented in this study. The appropriateness of this primary endpoint was also agreed with FDA during the pre-IND meeting.

The following hypotheses were tested to assess non-inferiority at a one-sided significance level of 2.5% (μ denotes the mean DSN under Neupogen (groups NEUZAR and NEU) and ZARXIO (groups ZAR and ZARNEU), respectively):

H_0: $\mu_{Neupogen} - \mu_{ZARXIO} \leq -1$ day
H_1: $\mu_{Neupogen} - \mu_{ZARXIO} > -1$ day

The planned sample size of 192 patients was derived to have at least 90% power to meet the primary objective of the study. The primary efficacy endpoint was analyzed using an analysis of covariance (ANCOVA) with the factors 'treatment group' and 'kind of chemotherapy' (strata: adjuvant, neoadjuvant) and the covariate 'baseline ANC', to calculate a one-sided 97.5% confidence interval (CI) for the difference in mean DSN between the two combined treatment groups in Cycle 1. Non-inferiority of ZARXIO was to be concluded if the lower limit of the one-sided 97.5% CI was larger than -1 day. The primary analysis of the main efficacy endpoint was based on the PP set, consisting of all patients who completed the first chemotherapy cycle without major protocol deviations, which constitutes the most conservative approach for a non-inferiority assessment.

In this study a difference of -1 day in the DSN in Cycle 1 was chosen as the largest acceptable difference of the test treatment compared to the reference treatment (non-inferiority margin) and was discussed and agreed with FDA during the pre-IND meeting in 09/2010. This margin was selected based on the fact that TAC chemotherapy is known to induce a median DSN of seven days in breast cancer patients receiving no G-CSF treatment (Nabholtz 2001), while G-CSF treatment reduces the mean DSN for this chemotherapy to 1.4 days (95% CI: 1.07 - 1.69) as shown in Amgen's pegfilgrastim (Neulasta®) Study 20020778 (Kaufman 2004). Therefore, a non-inferiority limit of -1 day preserves at least approximately 80% of the treatment effect of Neupogen. Moreover, in the studies conducted to support the approval of the long-acting rh-G-CSF Neulasta, a single dose of pegfilgrastim (Neulasta) was compared to daily administrations of filgrastim (Neupogen) with a non-inferiority margin of -1 day for the duration of grade 4 neutropenia in breast cancer patients treated with myelosuppressive chemotherapy (Holmes 2002, Green 2003). The same endpoint and margin was also used more recently in the pivotal study for tbo-filgrastim (Trade name: "Granix", Teva Pharmaceuticals) when assessing equivalence to Neupogen.

The endpoints of the study to assess similarity between ZARXIO and Neupogen are summarized below:

Efficacy:

Primary endpoint

- Mean DSN, defined as the number of consecutive days with Grade 4 neutropenia during Cycle 1

Secondary endpoints

- Incidence of FN, defined as oral temperature ≥ 38.3 °C while having an ANC < 0.5 x 109/L (both measured on the same day), by cycle and across all cycles
- Number of days of fever, defined as oral temperature ≥ 38.3 °C, for each cycle
- Depth of ANC nadir, defined as the patient's lowest ANC in Cycle 1
- Time to ANC recovery, defined as the time in days from the chemotherapy administration until the patient's ANC increases to ≥ 2 x 109/L after the nadir in Cycle 1
- Frequency of infections by cycle and across all cycles
- Incidence and duration of hospitalization due to FN

Safety:

- Incidence, occurrence, and severity of (serious) adverse events (AEs)
- Assessment of local tolerability at the injection site
- Systemic tolerance (physical examination and safety laboratory assessments)

Immunogenicity:

- Anti-rhG-CSF antibody formation

In addition to the primary analysis, the study was powered to show that alternating treatment with ZARXIO and Neupogen between chemotherapy cycles does not affect the efficacy, safety, and immunogenicity of filgrastim treatment. With respect to the interchangeability assessment, a sample size of 192 (96 switched, 96 un-switched) allowed the following analyses:

- Exclude an anti-rhG-CSF incidence rate > 3% with 95% certainty if no antibody positive cases are observed. 3% is the corresponding incidence listed in the European label (Summary of Product Characteristics) of Neupogen.
- Assess non-inferiority in overall FN rates between switched and un-switched patients based on a non-inferiority margin of 15% with 80% power. The non-inferiority margin of 15% was selected based on the fact that filgrastim treatment was known to reduce the incidence of FN by 20% to 30% as compared to no G-CSF support. The non-inferiority margin of 15% was thus to maintain at least 50% of the known effect size of filgrastim treatment.

Of note, the assessment of interchangeability is not the subject of the current submission.

7.3.3.2.1 Study results – Study Populations and Demographics

A total of 218 patients were randomized into the study of which 204 were included in the PP population for the primary analysis. A total of 34 patients did not complete the study as planned or discontinued study treatment prematurely. The patients were on average 49.0 years old with an overall range between 23 and 76 years. The mean duration since breast cancer diagnosis was 2.0 months (range from 0 to 171[1] months) and the majority of the patients had cancer of stage II (51.4%) or stage III (41.6%). A total of 163 patients (76.2%) were of ECOG status 0 and 51 patients (23.8%) were of ECOG status 1. Further details on the patient demographics are provided in Appendix 2 – Patient Demographics.

7.3.3.2.2 Study results – Primary Endpoint

The mean DSN in the PP population was approximately 1.2 days in both groups with an overall range of 0-4 days. The mean difference between ZARXIO and Neupogen was 0.04 days with a lower limit of the 97.5% CI of -0.26 days. ZARXIO is shown to be non-inferior to Neupogen, since the lower limit of the CI is well above the pre-defined non-inferiority margin of -1 day. The results from the full analysis set (FAS) confirm the conclusion based on the PP population.

Table 21 – Pivotal study EP06-302: Primary efficacy endpoint (mean DSN in Cycle 1)

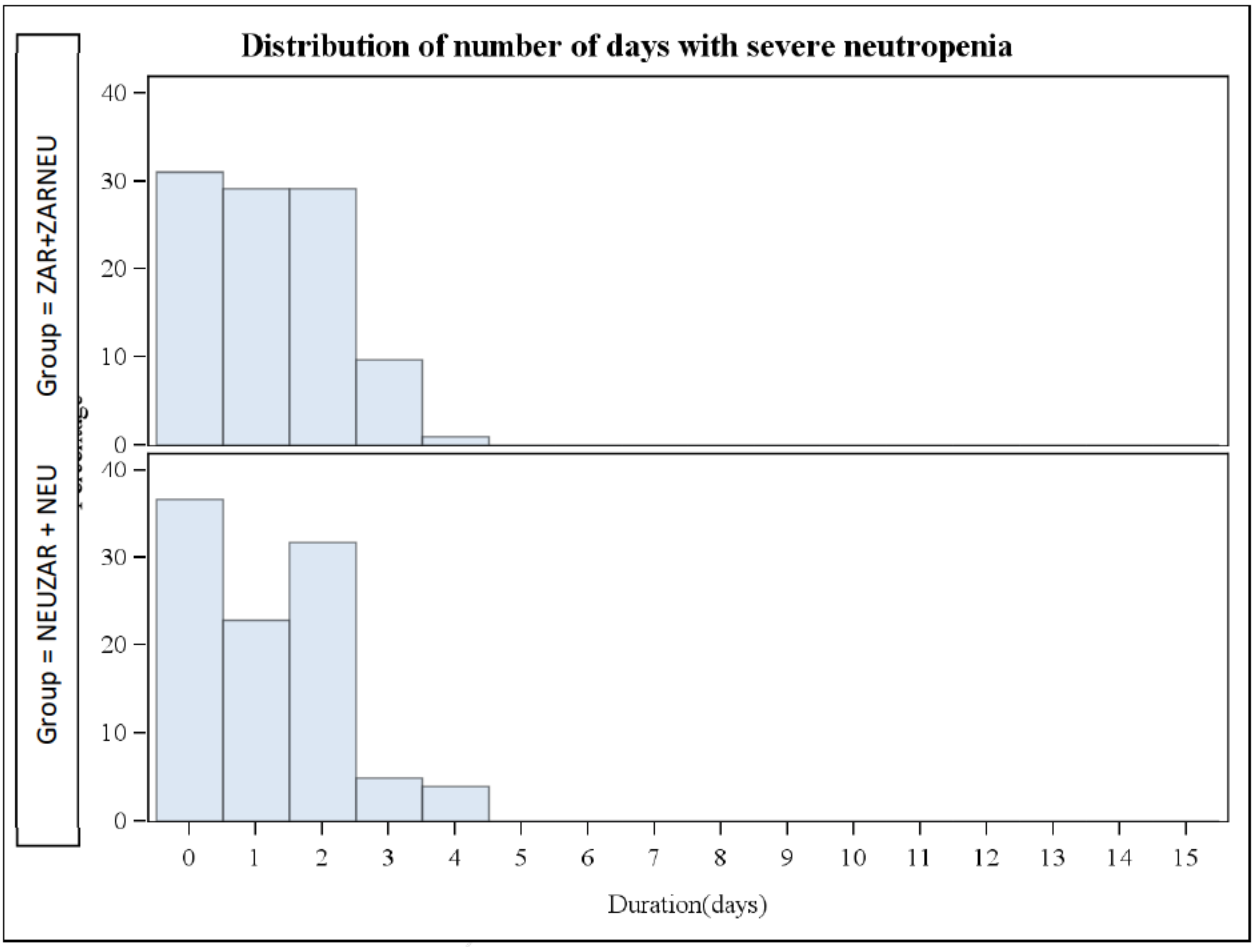
		PP population		**FAS population**	
		ZARXIO	**Neupogen**	**ZARXIO**	**Neupogen**
DSN in Cycle 1 (days)	**N**	101	103	107	107
	Mean	1.17	1.20	1.18	1.20
	SD	1.11	1.02	1.12	1.02
	Range	0-4	0-4	0-4	0-4
	Difference (97.5% CI)	0.04 (-0.26; ∞)		0.02 (-0.27; ∞)	
		N (%)	N (%)	**N (%)**	**N (%)**
DSN categories	**0-2 days**	92 (91.1)	92 (89.3)	96 (89.7%)	96 (89.7%)
	≥ 3 days	9 (8.9)	11 (10.7)	11 (10.3%)	11 (10.3%)

ZARXIO: Combination of groups ZAR+ZARNEU in Cycle 1; Neupogen: Combination of groups NEU+NEUZAR in Cycle 1; DSN: Duration of severe neutropenia; FAS – full analysis set; Note: Percentages are based on those patients with an available assessment (missing patients are not included) within the respective group.

The distribution of the number of days of severe neutropenia is depicted in Figure 31 for the PP population, which shows that an overall comparable picture with over 60% of the patients in each group experiencing at least one day of severe neutropenia.

[1] This patient was treated with adjuvant chemotherapy for a relapse of breast cancer. The operation was 1 month before randomization. However the initial diagnosis was 171 month before randomization.

Figure 31 – Distribution of the number of days of severe neutropenia in cycle 1 (PP population)



7.3.3.2.3 Study results – Secondary Endpoints

Febrile neutropenia

Febrile neutropenia (FN) was defined as oral temperature ≥ 38.3°C while having an ANC < 0.5 × 10^9/L (both measured on the same day). Incidences of FN in Cycle 1 as well as across all 6 cycles are shown below whereby for the cycle 1 analysis groups ZAR and ZARNEU as well as NEUZAR and NEU were combined to reflect the incidences for ZARXIO and Neupogen, respectively. For the analysis across all six cycles only those patients were considered who continuously received the respective product, explaining the lower number of patients included in the overall analysis. The incidences of FN were low in both groups, were not statistically significant, and were not clinically relevant both for cycle 1 as well as across all 6 cycles (Table 22).

Table 22 – Pivotal study EP06-302: FN incidence

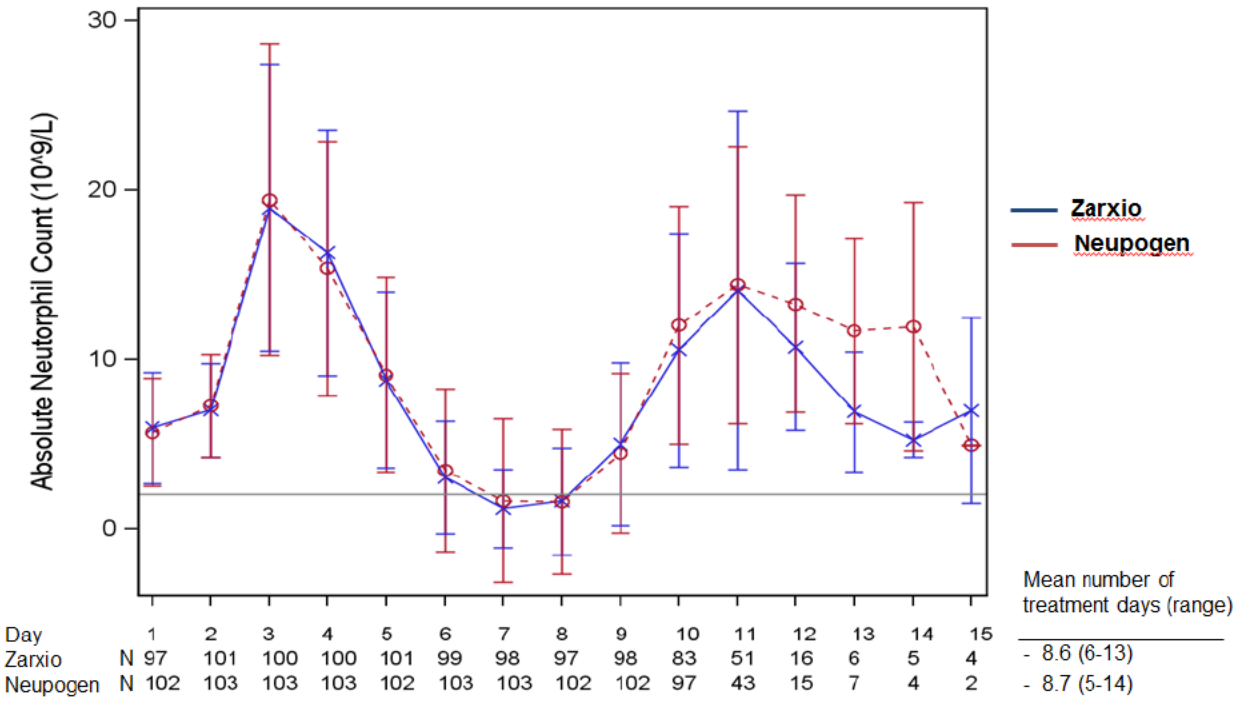
	Incidence of Febrile Neutropenia (FN) (PP Population)			
	Cycle 1		All 6 cycles	
FN	**ZARXIO**	**Neupogen**	**ZARXIO**	**Neupogen**
	N=101	N=103	N=40	N=46
At least one FN	4 (4.0%)	2 (1.9%)	2 (5.0%)	0 (0.0%)
95% CI for difference in FN rates	(-2.6%; 6.7%)		(-1.8%; 11.8%)	

ZARXIO: Combination of groups ZAR+ZARNEU in Cycle 1; Neupogen: Combination of groups NEU+NEUZAR in Cycle 1; FN: Febrile neutropenia; only patients continuously treated with either product are considered for the analysis across all 6 cycles

ANC time course in Cycle 1

The time course of the ANC in Cycle 1 is illustrated below in Figure 32. There were no marked differences in the mean ANC between ZARXIO and Neupogen up to Day 10. Following Day 10, when in most patients the ANC had recovered by reaching at least 10×10^9/L (ANC measurements were only made until the ANC recovered or until Day 15, whichever occurred first), the number of patients with measurements decreased markedly as the ANC recovered in more and more patients.

Figure 32 – Pivotal study EP06-302: Time course of ANC in Cycle 1 (PP set)



Time to ANC recovery in Cycle 1

A similar mean time to ANC recovery in Cycle 1 was observed for patients receiving ZARXIO (1.8 days ± 0.97 [SD]) and patients receiving Neupogen (1.7 days ± 0.81). Time to ANC recovery was defined as the number of days from the nadir until the ANC increased to ≥ 2 × 10^9/L. In both patient groups the time to ANC recovery ranged from 0 to 4.

Incidence of infections

Infections were identified as AEs coded in the system organ class (SOC) 'infections and infestations'. In Cycle 1, infections occurred in 2.0% and 1.9% of the patients treated with ZARXIO (combined groups ZAR and ZARNEU, N=101) and with Neupogen (combined groups NEU and NEUZAR, N=103), respectively (PP set). Across all cycles, infections occurred in 5.7% and 9.6% of patients treated with ZARXIO (group ZAR) and Neupogen (group NEU, n=52), respectively (safety set).

7.3.3.2.4 Study results – Switching

The assessment of the data generated from cycle 2 onwards ("switching data") demonstrated similar results for all treatment arms. In particular, the incidence of FN over all cycles was 2.3% in the un-switched and 6.7% in the switched groups. The lower limit of the 95% CI (-10.5%) for the difference between these groups did not exceed the non-inferiority margin of -15%, demonstrating that switching is not inferior to continuous treatment (PP-I set: all randomized patients who completed all six chemotherapy cycles without major protocol violations).

7.3.3.2.5 Study results – Conclusion

Non-inferiority between ZARXIO and US-licensed Neupogen was proven with respect to the duration of severe neutropenia in cycle 1 with the lower bound of the one-sided 97.5% confidence interval ((-0.26; ∞)) being well above the pre-defined margin of -1 day. The ANC profiles in cycle 1 were superimposable and the results of the secondary efficacy endpoints (incidence of FN, time to ANC recovery in Cycle 1, and incidence of infections) did not reveal substantial differences between the treatment arms and therefore corroborated the results of the primary endpoint.

No binding or neutralizing antibodies were observed throughout the study and the safety profiles of ZARXIO and Neupogen were similar as further discussed in Section 8.2.

7.3.3.3 Supportive studies

A single-arm clinical safety and efficacy study – EP06-301 was also conducted previously for the EU approval, which demonstrated comparable effectiveness of ZARXIO to published data with Neupogen. Details on the design and results of these studies can be found in Appendix 3 – Supportive Clinical Studies.

Preliminary effectiveness data are also available for an ongoing post-market study in Europe. The primary objective of this study is to investigate the safety profile of ZARXIO in healthy unrelated donors. The secondary objective is to assess the effectiveness of PBPC mobilization

with ZARXIO in terms of the $CD34^+$ cell count. Details on this study can also be found in Appendix 3 – Supportive Clinical Studies.

8 Comparison of clinical safety between ZARXIO and Neupogen

Two pivotal studies (the comparative efficacy and safety study EP06-302 in breast cancer patients undergoing myelosuppressive chemotherapy, and the PK/PD study EP06-109 in healthy volunteers) compared the safety profile of ZARXIO with that of the reference product US-licensed Neupogen.

Four supportive PK/PD studies in healthy volunteers provide comparative safety data with the EU-authorized Neupogen as comparator. Two non-comparative studies provide further relevant safety data: a comparative safety and efficacy study in breast cancer patients receiving myelosuppressive chemotherapy (EP06-301) and a post-authorization safety study (PASS) in healthy donors undergoing PBPC mobilization (EP06-501).

Immunogenicity of ZARXIO and Neupogen were also evaluated in these studies, with results provided and discussed in Section 8.4 of this document.

All studies showed that the safety profile of ZARXIO is similar to the well-established safety profile of Neupogen.

8.1 Safety population, evaluations and exposure

8.1.1 Key safety population

The safety evaluations in the two safety and efficacy studies EP06-302 and EP06-301 were performed during treatment with ZARXIO as prophylaxis of neutropenia in breast cancer patients receiving myelosuppressive chemotherapy. The safety population of these studies consisted of adult female patients with similar demographic and disease characteristics between treatment groups. However, different chemotherapy regimens were used in these two studies. Details on the patients' demographics are provided in Appendix 1 – PK and PD results from supportive PK/PD studies

The tables below summarize the results for the PK and PD parameters in the supportive PK/PD studies together with the respective confidence intervals for the comparison between ZARXIO and Neupogen.

Table 1 – Ratios and 90% confidence intervals for the PK parameters in the supportive PK/PD studies (PP populations)

				Geometric means			
Parameter	**Study**	**Dose (mcg/kg)**	**Route**	**ZARXIO**	**Neupogen***	**Ratio (%)**	**90% CI (%)**
AUC0-36h,sd	EP06-102	5	i.v.	632.1	634.2	99.68	[96.94; 102.47]
AUC0-24h,sd	EP06-105	1	s.c.	58.3	65.7	91.17	[85.95; 96.72]

Parameter	Study	Dose (mcg/kg)	Route	Geometric means		Ratio (%)	90% CI (%)
				ZARXIO	Neupogen*		
	EP06-103	2.5	s.c.	119.6	136.8	87.46	[80.95; 94.48]
	EP06-103	5	s.c.	370.3	383.7	95.87	[90.31; 101.78]
	EP06-101	10	s.c.	839.7	908.1	93.13	[88.76; 97.70]
AUC144-168h,ss	EP06-103	2.5	s.c.	42.6	49.1	86.14	[80.58; 92.08]
	EP06-103	5	s.c.	106.1	121.9	86.90	[80.90; 93.35]
	EP06-101	10	s.c.	175.3	193.1	90.78	[84.45; 97.60]
$Cmax_{,0\text{-}36h,sd}$	EP06-102	5	i.v.	186.4	188.7	98.82	[95.76; 101.98]
$Cmax_{,0\text{-}24h,sd}$	EP06-105	1	s.c.	9.1	10.6	88.50	[81.55; 96.03]
	EP06-103	2.5	s.c.	16.9	19.5	86.61	[78.63; 95.40]
	EP06-103	5	s.c.	48.2	49.9	95.91	[88.73; 103.67]
	EP06-101	10	s.c.	97.7	110.3	88.84	[82.49; 95.67]
$Cmax_{,144\text{-}216h,ss}$	EP06-103	2.5	s.c.	7.4	9.5	77.75	[70.81; 85.40]
	EP06-103	5	s.c.	21.8	27.2	80.05	[70.19; 91.30]
	EP06-101	10	s.c.	35.0	39.1	89.68	[81.83; 98.28]

*EU-authorized Neupogen; AUC = Area under the serum concentration-time curve between the specified time points; CI = Confidence interval; Cmax = Maximum observed serum concentration; Ratio: E2006/Neupogen; sd = single-dose; ss = steady state (multiple-dose)

Table 2 – AUEC of absolute neutrophil count and CD34+ cell counts across the supportive PK/PD studies (PP populations)

Study	Dose (mcg/kg)	Route	Parameter	Geometric means		Ratio (%)	95% CI (%)
				ZARXIO	Neupogen*		
ANC (109×h/L)							
EP06-105	1	s.c.	$AUEC0_{\rightarrow 120h}$	740.78	725.00	102.11	[96.68; 108.09]
EP06-103	2.5	s.c.	$AUEC0_{\rightarrow 216h}$	4224.0	4134.5	102.16	[99.49; 104.91]
EP06-103	5	s.c.	$AUEC0_{\rightarrow 216h}$	5191.8	5176.8	100.61	[98.01; 103.29]

EP06-101	10	s.c.	$AUEC0_{\rightarrow 216h}$	6474.5	6515.3	99.37	[96.30; 102.54]
EP06-102	5	i.v.	$AUEC0_{\rightarrow 120h}$	944.72	950.19	99.42	[93.52; 105.70]
CD34+ cell count (h×cells/µL)							
EP06-103	2.5	s.c.	$AUEC0_{\rightarrow 216h}$	2815.1	2694.0	104.49	[96.51; 113.14]
EP06-103	5	s.c.	$AUEC0_{\rightarrow 216h}$	2885.5	2898.3	98.99	[86.79; 112.90]
EP06-101	10	s.c.	$AUEC0_{\rightarrow 216h}$	5129.3	5023.3	102.11	[94.93; 109.83]

*EU-authorized Neupogen; AUEC = Area under the effect-time curve between the specified time points, Emax = Maximum observed effect; Ratio = ZARXIO/Neupogen

Appendix 2 – Patient Demographics

All PK/PD studies were conducted in adult healthy volunteers with similar demographic and baseline characteristics (see Appendix 2 – Patient Demographics).

8.1.2 Evaluations

Safety of ZARXIO was monitored through adverse event reporting, clinical laboratory testing, vital signs, physical examinations, and ECG. Immunogenicity was assessed in all studies in terms of monitoring for anti-rhG-CSF antibodies.

8.1.3 Exposure

Exposure data are available for the following studies and subject populations, data lock point 31-Jan-2014):

- Breast cancer patients in whom ZARXIO was given by the s.c. route (studies EP06-301 and EP06-302 (in this study vials were used))
- Healthy donors undergoing PBPC mobilization in whom ZARXIO was given by the s.c. route (study EP06-501)
- Healthy volunteers in whom ZARXIO was administered by the s.c. route (studies EP06-101, EP06-103, EP06-105, EP06-106, EP06-108, EP06-109 and EP06-110)
- Healthy volunteers in whom ZARXIO was given by the i.v. route (studies EP06-102 and EP06-107)

During the clinical development program, 28.6 patient-years exposure to ZARXIO was obtained in 334 breast cancer patients. Including the stem cell donors and the healthy volunteers in the PK/PD studies, 34.28 years exposure was obtained in 875 subjects (see Table 23).

Table 23 - Overall ZARXIO exposure in clinical studies by administration route

Treatment	N	Population	Days	Years[1]
ZARXIO s.c.	334	Breast cancer patients	10,440	28.6
ZARXIO s.c.[2]	295	Healthy volunteers	1029	2.82
ZARXIO s.c.	200	Healthy stem cell donors	1000	2.73
ZARXIO i.v.[3]	46	Healthy volunteers	46	0.13
ZARXIO overall exposure	875	Patients, healthy stem cell donors & healthy volunteers	12,515	34.28

[1] Years = days/365.25

[2] including PK/PD studies EP06-106, EP06-108 and EP06-110, conducted in Japanese healthy volunteers

[3] including PK/PD study EP06-107, conducted in Japanese healthy volunteers

8.1.3.1 Exposure to ZARXIO in patients by age group and gender

Exposure to ZARXIO in breast cancer patients in the two safety and efficacy studies (EP06-302 and EP06-301) is summarized in the following table.

Table 24 - Exposure to ZARXIO, by age group and gender (safety and efficacy studies)

Age group	Female patients (N)	Years

Age group	Female patients (N)	Years
≤ 18 years	No exposure available.	
> 18 ≤ 40 years	62	5.2
> 40 ≤ 65 years	242	20.9
> 65 years	30	2.5

Indication: Breast cancer patients receiving cytotoxic chemotherapy (studies EP06-302 and EP06-301); only female adult patients were included in these studies.

8.1.3.2 Exposure to ZARXIO by race

All pivotal and supportive clinical studies were conducted in Caucasian subjects, except for two black healthy volunteers in PK/PD study EP06-109.

Four PK/PD studies (EP06-106, EP06-107, EP06-108 and EP06-110) were conducted in 160 Japanese male healthy volunteers.

8.2 Safety results from clinical studies

8.2.1 Safety results from pivotal safety and efficacy study EP06-302

The most frequent treatment-emergent AEs that were reported in study EP06-302 in breast cancer patients are shown in Table 25, regardless of their relationship to treatment. The most frequent AEs were alopecia, nausea, asthenia, fatigue, and bone pain. The nature and frequency of the AEs were similar between the treatment arms.

Table 25 - Most frequent AEs by preferred MedDRA term regardless of relationship to treatment in EP06-302

Preferred term	ZARXIO (N=53)		ZARXIO-Neupogen (N=54)		Neupogen-ZARXIO (N=55)		Neupogen (N=52)	
	n (%)	#	n (%)	#	n (%)	#	n (%)	#
Alopecia	41 (77.4)	43	44 (81.5)	45	43 (78.2)	46	43 (82.7)	44
Nausea	29 (54.7)	103	33 (61.1)	95	32 (58.2)	116	37 (71.2)	149
Asthenia	20 (37.7)	66	28 (51.9)	98	32 (58.2)	111	28 (53.8)	101
Fatigue	17 (32.1)	52	9 (16.7)	17	11 (20.0)	38	13 (25.0)	38
Bone pain	13 (24.5)	33	20 (37.0)	46	19 (34.5)	60	19 (36.5)	60
Vomiting	9 (17.0)	27	10 (18.5)	22	10 (18.2)	21	9 (17.3)	19
Decreased appetite	8 (15.1)	21	4 (7.4)	8	3 (5.5)	6	13 (25.0)	32
Anemia	6 (11.3)	10	5 (9.3)	9	4 (7.3)	6	11 (21.2)	24
Pyrexia	6 (11.3)	9	3 (5.6)	8	1 (1.8)	1	1 (1.9)	2
Diarrhea	5 (9.4)	8	11 (20.4)	14	13 (23.6)	21	8 (15.4)	11
Neutropenia	5 (9.4)	21	7 (13.0)	18	6 (10.9)	20	6 (11.5)	22
Musculoskeletal pain	5 (9.4)	6	1 (1.9)	2	2 (3.6)	3	1 (1.9)	3
Erythema	5 (9.4)	15	2 (3.7)	3	6 (10.9)	19	6 (11.5)	23
Leukopenia	4 (7.5)	21	4 (7.4)	12	2 (3.6)	3	3 (5.8)	9
Abdominal pain	3 (5.7)	8	2 (3.7)	2	4 (7.3)	7	3 (5.8)	6
Arthralgia	3 (5.7)	8	4 (7.4)	4	6 (10.9)	8	3 (5.8)	3

Preferred term	ZARXIO (N=53)		ZARXIO-Neupogen (N=54)		Neupogen-ZARXIO (N=55)		Neupogen (N=52)	
	n (%)	#	n (%)	#	n (%)	#	n (%)	#
Febrile neutropenia	3 (5.7)	3	5 (9.3)	5	1 (1.8)	1	1 (1.9)	1
Stomatitis	3 (5.7)	3	3 (5.6)	3	0 (0.0)	0	2 (3.8)	4
Headache	3 (5.7)	6	3 (5.6)	3	2 (3.6)	2	1 (1.9)	1
Dizziness	3 (5.7)	8	0 (0.0)	0	3 (5.5)	4	1 (1.9)	1
Peripheral sensory neuropathy	3 (5.7)	3	0 (0.0)	0	2 (3.6)	2	1 (1.9)	1
Abdominal pain upper	2 (3.8)	3	0 (0.0)	0	5 (9.1)	10	2 (3.8)	2
Myalgia	2 (3.8)	5	3 (5.6)	4	3 (5.5)	5	3 (5.8)	4
Flushing	1 (1.9)	3	3 (5.6)	10	0 (0.0)	0	2 (3.8)	7
Hypothermia	0 (0.0)	0	3 (5.6)	6	1 (1.8)	14	1 (1.9)	1

Most frequent AEs: Incidence of at least 5% in any group

AEs are presented in descending order to the ZARXIO treatment group; n = number of patients with an event; # = Number of adverse events

ZARXIO-Neupogen: Patients who received ZARXIO in Cycle 1 and alternating treatment in cycles 2 to 6

Neupogen-ZARXIO: Patients who received Neupogen in Cycle 1 and alternating treatment in cycles 2 to 6

The AE profile in study EP06-302 is in-line with what can be expected for cancer patients receiving myelosuppressive chemotherapy, e.g. alopecia, nausea, asthenia, fatigue and vomiting, or for the rhG-CSF product class, e.g. bone pain or musculoskeletal pain. The frequency of bone/musculoskeletal is comparable between ZARXIO and Neupogen and lies in the range of what is described for the rhG-CSF product class (Neupogen SmPC UK 2014).

Serious adverse events

Fourteen SAEs were reported in 12 patients (5.6% of patients) including one death. The death was due to pulmonary embolism considered not related to study drug, but suspected to be related to breast cancer and chemotherapy. It was a 61-year-old breast cancer patient who died due to thromboembolism of the pulmonary artery. The patient's medical history included rheumatic heart disease, hypertension, left atrial dilation, moderate heart failure, and atherosclerosis of aorta, coronary arteries and retroperitoneal arteries. Seven days after receiving TAC chemotherapy in Cycle 1 and with a total exposure to ZARXIO of 6 days the patient was hospitalized with the diagnosis of thromboembolism of the pulmonary artery branches. The patient died on the same day.

None of the SAEs were considered to be drug-related. Slightly more SAEs were reported in the ZARXIO group (6 events in 5 patients) as compared to the Neupogen (US-licensed) group (3 events in two patients). One AE led to study discontinuation (blood pressure fluctuation in a patient treated with Neupogen). A summary of serious and significant AEs in study EP06-302 is presented in Table 26.

Table 26 - Summary of serious and significant adverse events in EP06-302

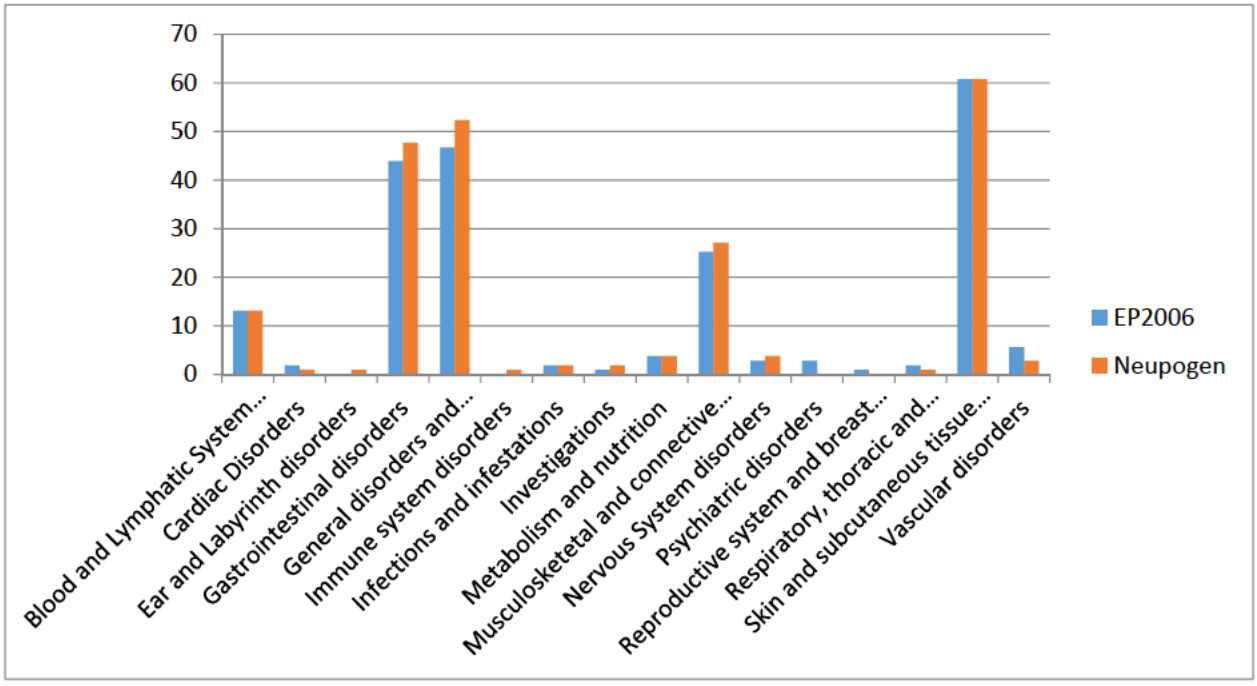
	ZARXIO (N=53)	ZARXIO–Neupogen (N=54)	Neupogen-ZARXIO (N=55)	Neupogen (U.S.-licensed) (N=52)

Primary system organ class Preferred term	n (%)	#	n (%)	#	n (%)	#	n (%)	#
Number of patients with any SAE	5 (9.4)	6	4 (7.4)	4	1 (1.8)	1	2 (3.8)	3
Blood and lymphatic system disorders	3 (5.7)	4	4 (7.4)	4	1 (1.8)	1	2 (3.8)	2
Febrile neutropenia	3 (5.7)	3	4 (7.4)	4	1 (1.8)	1	1 (1.9)	1
Anemia	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	1 (1.9)	1
Leukopenia	1 (1.9)	1	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Gastrointestinal disorders	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	1 (1.9)	1
Diarrhea	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	1 (1.9)	1
Vascular disorders	2 (3.8)	2	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Embolism	1 (1.9)	1	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Hypertensive crisis	1 (1.9)	1	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Number of patients with any AE leading to study disc./withdrawal	1 (1.9)	1	0 (0.0)	0	1 (1.8)	1	0 0.0)	0
Vascular disorders	1 (1.9)	1	0 (0.0)	0	1 (1.8)	1	0 (0.0)	0
Blood pressure fluctuation	0 (0.0)	0	0 (0.0)	0	1 (1.8)	1	0 (0.0)	0
Embolism	1 (1.9)	1	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0

AE = Adverse event; n = Number of patients in a treatment group; # = Number of adverse events; SAE = Serious adverse event

As seen in Figure 33, no differences were observed in AEs by system organ class in cycle 1, which is the most important cycle from a clinical perspective, and in which we have more sample size than in subsequent cycles (based on study design).

Figure 33 - Adverse events in Cycle 1 of Study 302 by System Organ Class



8.2.2 Safety results from pivotal PK/PD study EP06-109

In the PK/PD study EP06-109 no SAE occurred. None of the AEs was severe, and there was no discontinuation due to an AE. Increased orthostatic heart rate response, headache, and back pain were most frequently documented as AEs in both treatment groups, regardless of their relationship to treatment. AEs with a frequency of at least 5% in any treatment group are presented in Table 27. The nature and frequency of the AEs in study EP06-109 were similar between the treatment arms.

Table 27 - AEs by preferred term regardless of relationship to treatment (≥5% in any group) in EP06-109

Preferred term	ZARXIO (N=27)		Neupogen (N=27)	
	n (%)	#	n (%)	#
Orthostatic heart rate response increased	5 (18.5)	8	4 (14.8)	6
Headache	3 (11.1)	3	3 (11.1)	3
Back pain	3 (11.1)	3	2 (7.4)	2
Injection site erythema	2 (7.4)	2	0 (0.0)	0
Vessel puncture site hematoma	2 (7.4)	3	0 (0.0)	0
Injection site hematoma	1 (3.7)	1	2 (7.4)	2
Myalgia	0 (0.0)	0	2 (7.4)	2
Upper respiratory tract infection	0 (0.0)	0	2 (7.4)	2

AEs are presented in descending order to the ZARXIO treatment group; n = number of subjects with an event; # = Number of adverse events

8.2.3 Safety results from supportive safety and efficacy study EP06-301

The protocol of this single-arm safety and efficacy study identified five AEs as being of special interest because they are commonly reported during treatment with G-CSF. These AEs were referred to as "rhG-CSF associated adverse events" and consist of musculoskeletal pain and elevations of lactic dehydrogenase (LDH), alkaline phosphatase, serum uric acid and AST. For these 5 AEs, 39 (23%) patients reported 89 events. A relationship to ZARXIO was suspected for 19 (11%) patients (44 events). All of these events were mild (34 [20%] patients, 79 events) or moderate (7 [4%] patients, 10 events) in intensity.

Serious adverse events

In study EP06-301 20 patients reported 23 SAEs, including 3 death cases which are described below. The SAEs other than death included febrile neutropenia (14 patients), diarrhea (2 patients) and anemia, atrial fibrillation, hepatitis, hypertensive crisis (1 patient each).

Three patients (1.8%) in study EP06-301 discontinued because of AEs: two patients discontinued study chemotherapy due to SAEs (febrile neutropenia, atrial fibrillation) and one patient due to a non-serious AE (allergic dermatitis). A relationship to ZARXIO was not suspected for any SAE or discontinuation due to AE.

A summary of SAEs and AEs leading to discontinuations in study EP06-301 is presented in Table 28.

Table 28 - Summary of serious and significant adverse events in study EP06-301

	Number of patients	Number of events

	Number of patients	Number of events
	n (%)	#
Patients studied	170 (100)	1583
Total number of patients with SAEs	20 (11.8)	23
Serious or significant events		
Death	3 (1.8)	3
Other SAEs	19 (11.2)	20
Discontinued due to SAEs	2 (1.2)	2
Discontinued due to AEs	1 (0.6)	1

SAE = Serious adverse event

Three patients died during the follow-up period, two due to disease progression (both patients had advanced breast cancer at screening) and one due to injuries resulting from a car accident. A relationship to ZARXIO or to study chemotherapy was not suspected for any of these events.

8.2.4 Safety results from supportive PK/PD studies

No SAEs were reported in any of the PK/PD studies EP06-101, EP06-102, EP06-103 and EP06-105. Withdrawals of healthy volunteers due to AEs were uncommon in the supportive PK/PD studies: 2 (5.0%) in study EP06-101 (influenza, pregnancy), 2 (7.7%) in study EP06-102 (headache/nausea/vomiting, viral infection), none in studies EP06-103 and EP06-105.

In studies EP06-101, EP06-102 and EP06-105 no severe AE occurred, in study EP06-103 1.4% of the AEs in the ZARXIO treatment groups were severe (Neupogen treatment groups: 3.6%).

Study EP06-101

Most drug-related AEs were “musculoskeletal and connective tissue disorders” and “nervous system disorders” (

Table 29). Back pain was the most frequent AE, with a total of 54 events (23.3%; ZARXIO: 25 events, 22.1%; Neupogen: 29 events, 24.4%). Headache was the second most frequent AE, with a total of 57 events (24.6%; ZARXIO: 31 events, 27.4%; Neupogen: 26 events, 21.8%).

Table 29 - Most frequent (>5%) drug-related adverse events in EP06-101 summarized by preferred term, treatment, and severity

Preferred term	Total number of drug-related AEs	Treatment	Severity		
			Mild	Moderate	Severe
Overall	100	ZARXIO	83 (83.0%)	17 (17.0%)	0 (0.0 %)
	94	Neupogen	76 (80.9%)	18 (19.1%)	0 (0.0 %)
Headache	31	ZARXIO	27 (87.1%)	4 (12.99%)	0 (0.0 %)
	26	Neupogen	19 (73.1%)	7 (26.9%)	0 (0.0 %)
Back pain	25	ZARXIO	23 (92%)	2 (8.0%)	0 (0.0 %)
	29	Neupogen	25 (86.2%)	4 (13.8%)	0 (0.0 %)
Pain in extremity	12	ZARXIO	11 (91.7%)	1 (8.3%)	0 (0.0 %)
	16	Neupogen	16 (100.0%)	0 (0.0 %)	0 (0.0 %)
Chest wall pain	5	ZARXIO	5 (100.0%)	0 (0.0 %)	0 (0.0 %)
	6	Neupogen	5 (83.3%)	1 (16.7%)	0 (0.0 %)
Arthralgia	6	ZARXIO	6 (100.0%)	0 (0.0 %)	0 (0.0 %)
	3	Neupogen	3 (100.0%)	0 (0.0 %)	0 (0.0 %)

Study EP06-102

Most drug-related AEs were “nervous system disorders“, “general disorders and administration site conditions” and “musculoskeletal and connective tissue disorders” (

Table 30). Back pain and headache were the most frequent drug-related AEs. Headache was the most frequent AE, with 19 events (23.2%; ZARXIO: 10 events, 34.3%; Neupogen: 9 events, 23.7%). Fatigue (general disorders and administration site conditions), was the second most frequent AE, with 9 events (11.0%; ZARXIO: 4 events, 11.4%; Neupogen: 5 events, 13.2%). Blood and lymphatic disorders, gastrointestinal disorders, laboratory parameters, reproductive system and breast disorders, skin and subcutaneous tissue disorders, and vascular disorders were reported as drug-related AEs considerably less frequently.

Table 30 - Most frequent (>5%) drug-related adverse events in EP06-102 by preferred term, treatment, and severity

Preferred term	Total number of AEs n (%)	Treatment	Severity		
			Mild	Moderate	Severe
Overall	31	ZARXIO	31 (100.0%)	0 (0.0 %)	0 (0.0 %)
	35	Neupogen	32 (91.4%)	3 (8.6%)	0 (0.0 %)
Headache	10	ZARXIO	10 (100.0 %)	0 (0.0 %)	0 (0.0 %)
	9	Neupogen	8 (88.9 %)	1 (11.1 %)	0 (0.0 %)
Fatigue	4	ZARXIO	4 (100.0 %)	0 (0.0 %)	0 (0.0 %)
	5	Neupogen	5 (100.0 %)	0 (0.0 %)	0 (0.0 %)
Bone pain	3	ZARXIO	3 (100.0 %)	0 (0.0 %)	0 (0.0 %)
	3	Neupogen	2 (66.7 %)	1 (33.3 %)	0 (0.0 %)

Study EP06-103

The majority of reported AEs were considered drug-related. Most drug-related AEs were “investigations“, “musculoskeletal and connective tissue disorders” and “nervous system

disorders“ (Table 31). Thus, increased LDH, increased alkaline phosphatase and headache were the most frequent drug-related AEs. Frequencies for drug-related AEs were similar for the two treatments on all levels of severity.

Table 31 - Most frequent (>5%) drug-related adverse events in Study EP06-103 summarized by preferred term, treatment, and severity

Dose Group 1 (2.5 µg/kg)					
Preferred term	**Total number of drug-related AEs**	**Treatment**	**Severity**		
			Mild	**Moderate**	**Severe**
Overall	145	ZARXIO	62 (42.8%)	81 (55.9%)	2 (1.4%)
	154	Neupogen	65 (42.2%)	88 (57.1%)	1 (0.6%)
LDH increased	22	ZARXIO	0 (0.0%)	22 (100.0%)	0 (0.0%)
	21	Neupogen	0 (0.0%)	21 (100.0%)	0 (0.0%)
Blood alkaline phosphatase increased	25	ZARXIO	0 (0.0%)	25 (100.0%)	0 (0.0%)
	23	Neupogen	0 (0.0%)	23 (100.0%)	0 (0.0%)
Headache	24	ZARXIO	21 (87.5%)	3 (12.5%)	0 (0.0%)
	29	Neupogen	21 (72.4%)	8 (27.6%)	0 (0.0%)
Back pain	15	ZARXIO	7 (46.7%)	8 (53.3%)	0 (0.0%)
	10	Neupogen	8 (80.0%)	2 (20.0%)	0 (0.0%)
Blood uric acid increased	12	ZARXIO	1 (8.3%)	11 (91.7%)	0 (0.0%)
	13	Neupogen	0 (0.0%)	13 (100.0%)	0 (0.0%)
Myalgia	6	ZARXIO	3 (50.0%)	2 (33.3%)	1 (16.7%)
	9	Neupogen	7 (77.8%)	3 (33.3%)	0 (0.0%)
Bone pain	3	ZARXIO	3 (100.0%)	0 (0.0%)	0 (0.0%)
	9	Neupogen	6 (66.7%)	3 (33.3%)	0 (0.0%)
Platelet count decreased	3	ZARXIO	0 (0.0%)	3 (100.0%)	0 (0.0%)
	6	Neupogen	0 (0.0%)	6 (100.0%)	0 (0.0%)
Dose Group 2 (5 µg/kg)					
Overall	177	ZARXIO	80 (45.2%)	97 (54.8%)	0 (0.0%)
	167	Neupogen	61 (36.5%)	101 (60.5%)	5 (3.0%)
Headache	34	ZARXIO	23 (67.6%)	11 (32.4%)	0 (0.0%)
	26	Neupogen	12 (46.2%)	12 (46.2%)	2 (7.7%)
Blood alkaline phosphatase	27	ZARXIO	0 (0.0%)	27 (100.0%)	0 (0.0%)
increased	28	Neupogen	0 (0.0%)	28 (100.0%)	0 (0.0%)
LDH increased	26	ZARXIO	0 (0.0%)	26 (100.0%)	0 (0.0%)
	25	Neupogen	0 (0.0%)	25 (100.0%)	0 (0.0%)
Back pain	26	ZARXIO	22 (84.6%)	4 (15.4%)	0 (0.0%)
	26	Neupogen	17 (65.4%)	9 (34.6%)	0 (0.0%)
Blood uric acid increased	9	ZARXIO	0 (0.0%)	9 (100.0%)	0 (0.0%)
	9	Neupogen	0 (0.0%)	9 (100.0%)	0 (0.0%)
Pain in extremity	3	ZARXIO	2 (66.7%)	1 (33.3%)	0 (0.0%)

	8	Neupogen	6 (75.0%)	1 (12.5%)	1 (12.5%)
Dizziness	5	ZARXIO	4 (80.0%)	1 (20.0%)	0 (0.0%)
	5	Neupogen	4 (80.0%)	0 (0.0%)	1 (20.0%)
Fatigue	4	ZARXIO	4 (100.0%)	0 (0.0%)	0 (0.0%)
	7	Neupogen	4 (57.1%)	2 (28.6%)	1 (14.3%)

Study EP06-105

Headache was reported as the most frequent drug-related adverse event with 13 events (ZARXIO: 8 events, Neupogen: 5 events). Bone pain was the second most frequent drug-related adverse event with 3 events observed in subjects treated with Neupogen (Table 32).

Table 32 - Most frequent (>5%) drug-related adverse events in EP06-105 summarized by preferred term, treatment and severity

Preferred term	Total number of drug related AEs	Treatment	Severity		
			Mild	Moderate	Severe
Overall	8	ZARXIO	8 (100.0%)	0 (0.0%)	0 (0.0%)
	8	Neupogen	6 (75.0%)	2 (25.0%)	0 (0.0%)
Bone pain	0	ZARXIO	0 (0.0%)	0 (0.0%)	0 (0.0%)
	3	Neupogen	3 (100.0%)	0 (0.0%)	0 (0.0%)
Headache	8	ZARXIO	8 (100.0%)	0 (0.0%)	0 (0.0%)
	5	Neupogen	3 (60.0%)	2 (40.0%)	0 (0.0%)

8.2.5 Safety results from supportive post-authorization safety study EP06-501

In the ongoing study EP06-501 the most frequent AEs during the mobilization period were bone pain (108/118, 86.0%); blood potassium decreased (24/28, 19.8%); and back pain (15/118, 12.4%). All events of decreased blood potassium were considered related to the apheresis procedures and not to study drug by the investigators. A summary of AEs in study EP06-501 is presented in Table 33.

Table 33 - Summary of adverse events in EP06-501

	During mobilization	After mobilization
No. of healthy donors, N (%)	121 (100.0)	121 (100.0)
No. of donors with AEs, n (%)	118 (97.5)	26 (21.5)
Relationship to study drug, n (%)	115 (95.0)	3 (2.5)
Severity of related AEs, n (%)		
Mild	24 (20.9)	1 (33.3)
Moderate	52 (45.2)	1 (33.3)
Severe	39 (33.9)	1 (33.3)
AEs related to apheresis N (%)	30 (24.8)	0 (0.0)

AE = Adverse event

As per cut-off date 28-Aug-2013, three SAEs occurred: "chest pain", "chest pain plus dyspnea", and "thyroid neoplasm" [benign neoplasm according to histological workup] in three donors. These SAEs resolved completely and none of the donors experienced adverse events requiring transient or permanent premature discontinuation of the mobilization treatment with ZARXIO.

8.2.6 Clinical chemistry, hematology, urinalysis

Laboratory parameters showing a marked change from baseline were reported as AEs.

As is well described for filgrastim treatment, some laboratory parameters (e.g. serum alkaline phosphatase, lactic dehydrogenase, serum uric acid) (Amgen USA 2014) increased transiently. No clinically relevant or lasting changes in laboratory parameters were observed in the PK/PD studies. The laboratory findings from the two safety and efficacy studies in breast cancer patients undergoing myelosuppressive chemotherapy were consistent with what could be expected in that patient population, as summarized below.

Pivotal safety and efficacy study EP06-302

Due to the nature of the cytotoxic chemotherapy, clinically significant abnormalities were mainly decreases in hematology parameters in each cycle, most frequently white blood cells as well as hemoglobin and platelets. The mean changes in these hematology parameters were not different between ZARXIO and Neupogen.

There was no clinically relevant trend observed in clinical chemistry parameters.

In conclusion, laboratory parameters in the ZARXIO and Neupogen treatment groups were comparable.

Supportive safety and efficacy study EP06-301

All patients had at least one hematological abnormality reflective of the cytotoxicity associated with chemotherapy.

In addition to the hematology findings, a number of patients were reported with changes in chemistry laboratory parameters, which were consistent with patients receiving cytotoxic chemotherapy.

Supportive study in healthy stem cell donors (EP06-501)

In accordance with the mode of action of filgrastim, white blood cell count increased substantially during mobilization and normalized afterwards. Increases in chemical chemistry parameters were also transient.

8.3 Conclusions on the clinical safety results

The safety profile of ZARXIO was similar to that of US-licensed Neupogen in cancer patients receiving myelosuppressive chemotherapy and in healthy volunteers. In the pivotal safety and efficacy study EP06-302, none of the observed SAEs was considered to be related to the study drug. The most frequent related adverse events (e.g. bone pain is reflective of the pharmacodynamic effect of filgrastim.)

Safety and tolerability of ZARXIO as prophylaxis for neutropenia in cancer patients receiving myelosuppressive chemotherapy was also shown in the supportive uncontrolled safety and efficacy study EP06-301.

No SAEs in any healthy volunteer were observed in the PK/PD studies. In the pivotal PK/PD study EP06-109, none of the reported AEs was categorized as severe, significant, or serious.

There were no concerning or unexpected safety findings in the ongoing PASS EP06-501 in healthy stem cell donors.

No subject in any study developed anti-rhG-CSF antibodies following administration of ZARXIO (refer to Section 8.5.2).

In all studies the results from the laboratory tests, vital signs measurements and physical examinations showed no clinically relevant changes considered to be related to ZARXIO. The positive safety experience from the clinical studies has been confirmed by post-marketing pharmacovigilance data in the EU and other countries (see Section 8.4).

8.4 Post-marketing experience (Ex-US)

Filgrastim-containing products from Sandoz and partner companies are licensed in all countries of the European Economic Area (30 full member states, 1 provisional member state) and have been on the market since 2009. Further, Sandoz and partner companies received 32 additional approvals worldwide. European post-marketing experience represents approximately 7 million patient-days of exposure. This significant experience with the product results in a deep understanding of the safety profile of the product. Sandoz closely monitors the safety of the product on the market worldwide and summary reports of the post-marketing safety are generated in the form of periodic safety update reports (PSURs) on a consistent basis. Post-marketing data arises through spontaneous case reporting, non-interventional post-marketing studies and published literature.

During the period covered by this review (06 Feb 2009 – 31 Jul 2014), no actions (other than updates to the reference product label) were taken for safety reasons by Regulatory Authorities or the Marketing Authorization Holder regarding:

- Marketing authorization withdrawal, revocation or suspension
- Failure to obtain a marketing authorization renewal

- Failure to obtain an authorization for a clinical trial
- Clinical trial suspension (partial, complete or early termination)
- Recall of investigational drug or comparator

Incidences of known side effects of the G-CSF product class arising from the safety and efficacy trials and post-marketing experience are consistent with what is included in the Neupogen label.

In summary:

- The biosimilar products Zarzio/Filgrastim Hexal have been marketed since September 2009.
- The adverse effect reports submitted in association with Zarzio/Filgrastim Hexal over the period of review for this renewal have not revealed any new important safety signals of concern.
- The post-marketing experience has not revealed any significant changes in the safety profile of Zarzio/Filgrastim Hexal compared to the reference product.
- The overall safety profile of Zarzio/Filgrastim Hexal products since the original marketing authorisation was granted remains unchanged.

This is confirmed by a publication of Gascon et al, which examined the safety profiles of different biosimilar filgrastim products and concluded that statistically no one product is more or less safe (Gascon 2013). Overall, it can be concluded that according to post-marketing data, the safety profiles of ZARZIO and Neupogen are similar. The benefit/risk assessment is considered favorable.

8.5 Immunogenicity

The emergence of antibodies to human recombinant proteins is well documented. Antibodies directed against a therapeutic agent may have neutralizing activity and interfere with the efficacy of treatment. However, there has been no evidence of neutralizing antibodies with Neupogen (Amgen USA 2014). Screening for anti-rhG-CSF antibodies was included in all preclinical and clinical study designs of the ZARXIO program.

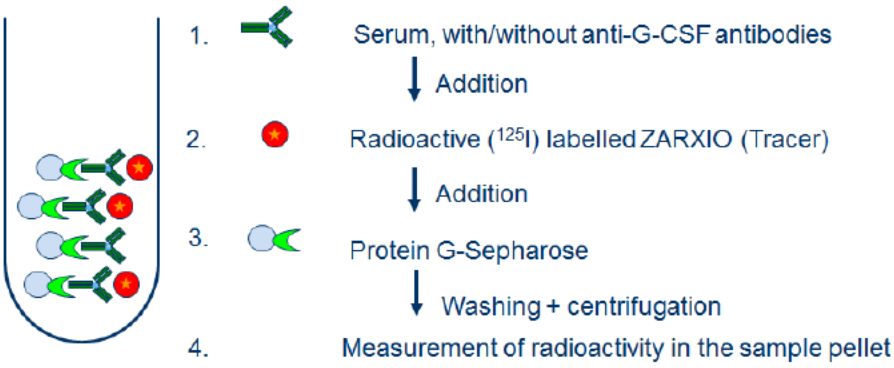
8.5.1 Immunogenicity assay design, assay sensitivity and specificity

The immune response after rhG-CSF administration in clinical studies was evaluated by a three-step procedure comprising a validated binding radioimmunoprecipitation (RIP) and a validated neutralization antibody assay (NAB).

The screening and the confirmatory (specificity) RIP assay for the detection of anti-rhG-CSF antibodies was developed and validated according to international guidelines. Briefly, standards, quality controls and study samples were incubated with [^{125}I]-labeled rhG-CSF tracer. After binding equilibrium was reached between antibodies and antigens, the formed immune-complexes were precipitated with Protein-G sepharose®. Unbound tracer was washed away and the precipitated amount of radioactivity was measured using a gamma counter.

Figure 34 summarizes the RIP assay principle for the detection of anti-rhG-CSF antibodies.

Figure 34 - RIP assay principle for the detection of anti-rhG-CSF



Study samples were first analyzed in a screening assay which included a calibration curve of reference item and two QC sets. Serum samples with a result above the screening cut-point value were analyzed in the confirmatory assay to prove the specificity of the anti-rhG-CSF antibodies detected in the screening assay. For this, excessive amount of unlabeled rhG-CSF was added to samples above the screening assay cut-point value. Specific anti-rhG-CSF antibodies bind to unlabeled rhG-CSF, leading to a reduction in assay read-out from the spiked compared to the unspiked samples. Test samples in which the average assay signal decreased the pre-determined specificity cut-point value after adding excess of unlabeled rhG-CSF were considered to contain specific anti-rhG-CSF antibodies and reported as "positive" for anti-rhG-CSF antibodies

The binding RIP assay is capable to detect anti-rhG-CSF antibodies in serum samples from healthy individuals as well as from breast cancer patients. The assay sensitivity (in 100% human serum) was determined to be 14.1 ng/mL for healthy individual serum and 9.5 ng/mL for breast cancer serum. The assay has an adequate tolerance to drug interference, at low and high concentration of positive controls antibodies at least a 50-fold excess of drug was tolerated. To demonstrate that the assay detects anti-rhG-CSF antibodies directed against ZARXIO and US-licensed Neupogen specifically, positive controls were tested in the confirmatory/ specificity assay with a range of spiked drug concentrations of ZARXIO and US-licensed Neupogen; the resulting depletion curves were superimposable, demonstrating no differences between both products.

The determination of the assay positivity cut-points (screening and confirmatory (specificity) assay cut-points) was performed in pre-study validations using samples representing the specific study populations. In case the determined screening cut-point couldn't be applied i.e. due to a different matrix background as in study EP06-109, study specific screening cut-points, using pre-dose samples were determined and used. For study EP06-302 the screening cut-point determined within the pre-study validation was applied, as predose samples were found not representative for post-dose sampling time points. Due to the chemotherapy co-medication the overall number of initial screening positive samples in this study was found to be lower compared to study EP06-109. In both studies no samples were confirmed positive for anti-rhG-CSF antibodies.

Samples positive for binding antibodies in the confirmatory (specificity) RIP assay were further evaluated for neutralizing anti-rhG-CSF antibodies in a cell-based NAB assay. The NAB assay

was developed based on the inhibition of G-CSF-induced proliferation of NFS-60 cells a mouse myelogenous leukemia cell line. G-CSF stimulates NFS-60 cells to proliferate; in the presence of serum containing neutralizing antibodies, the proliferation was inhibited. Proliferation was measured the by quantifying intracellular ATP using a luciferase chemiluminescence system. The measured luminescent signal was proportional to the amount of ATP, which was directly proportional to the number of cells present.

8.5.2 Immunogenicity testing in the ZARXIO clinical program

The emergence of antibodies to human recombinant proteins is well documented. Antibodies directed against a therapeutic agent may have neutralizing activity and interfere with the efficacy of treatment. While there has been no evidence of neutralizing antibodies with Neupogen, screening for anti-rhG-CSF antibodies was included in all study designs of the ZARXIO program, except for the study EP06-501. Table 34 summarizes all studies that included immunogenicity assessments.

Table 34 - Summary of studies including immunogenicity assessment of ZARXIO

Study	Design	Study population	Dose and regimen	Immunogenicity sampling
Pivotal studies				
EP06-302	Randomized, double-blind, multi-center safety and efficacy study	218 breast cancer patients receiving six cycles of TAC chemotherapy	EP2006 and U.S.-licensed Neupogen (supplied in vials, 480 mcg in 1.6 mL), s.c., daily dose of 5 mcg/kg, from Day 2 of each chemotherapy cycle until the ANC recovered to 10×109/L after the nadir or up to a maximum of 14 days (whichever occurred first)	anti-rhG-CSF antibody formation was analyzed prior to the first injection of study drug in Cycle 1, on Day 1 of each subsequent cycle, at the end of treatment (or early termination) visit, and at the follow-up visit six weeks after the start of the last chemotherapy cycle (approximately four weeks after the last study drug administration).
EP06-109	Randomized, double-blind, two-way crossover	28 healthy volunteers	Single s.c. dose of EP2006 and Neupogen (U.S.-licensed): 10 mcg/kg/day	Antibody formation against rhG-CSF (EP2006 and US-licensed Neupogen) was investigated by measuring anti-rhG-CSF antibodies in all 28 healthy volunteers at three times: 0.5 hour pre-dose in Periods 1 and 2 and after study drug dosing (at the follow-up visit).

Study	Design	Study population	Dose and regimen	Immunogenicity sampling
Supportive controlled studies				
EP06-101	Randomized, double blind, two-way crossover	40 healthy volunteers	Multiple s.c. doses of EP2006 and Neupogen (EU-authorized): 10 mcg/kg/day	Serum samples for antibody analysis were taken at baseline (screening), one hour before start of the treatment period II (28 days after treatment period I) and at the follow-up visit (Day 70).
EP06-102	Randomized, double blind, two-way crossover	26 healthy volunteers	Single i.v. dose of EP2006 and Neupogen (EU-authorized): 5 mcg/kg/day)	Serum samples for antibody analysis were taken at baseline (screening), one hour before the start of treatment period II (14-21 days after treatment period I) and at follow-up (8-15 days after treatment period II).
EP06-103	Randomized, double-blind, two-way crossover, with two dose groups	56 healthy volunteers	Multiple s.c. applications of EP2006 and Neupogen (EU-authorized) in two different doses: 2.5 and 5 mcg/kg/day	Serum samples for antibody analysis were taken at baseline (screening), one hour before the start of treatment period II, and at follow-up (at day 18 -33 after final study period II).
EP06-105	Randomized, double blind, two-way crossover	24 healthy volunteers	Single s.c. dose of EP2006 and Neupogen (EU-authorized): 1 mcg/kg/day	Serum samples for antibody analysis were taken one hour before the first administration on study day one of each study period and at the follow-up visit (at day 8 -15 after final study period II).
Supportive uncontrolled studies				
EP06-301	Open-label, single-arm, multi-center safety and efficacy study	170 breast cancer patients receiving four cycles of AT chemotherapy	EP2006 (pre-filled syringes), s.c., daily dose of 5 mcg/kg, from Day 2 of each chemotherapy cycle until the ANC recovered to 10×10^9/L after the nadir or up to a maximum of 14 days (whichever occurred first).	Serum samples for antibody analysis were taken at baseline (screening) or at Day 1 of Cycle 1 (C1D1), at Day 1 of Cycle 2 (C2D1), at Day 21 of Cycle 4 (C4D21) and at study termination (Day 91).

In total, 2724 serum samples were tested for binding anti-rhG-CSF antibodies in clinical studies within the ZARXIO program. All samples were tested with the screening RIP assay and 34 were analyzed with the confirmatory (specificity) RIP assay. From these 34 serum samples, three

samples were tested positive for binding antibodies. These three samples belonged to a subject from study EP06-102 and included the sample taken before study drug application. Thus, the result in the RIP assay was positive already at baseline; in addition, no increase was detected during treatment and no neutralizing antibodies were detected in these serum samples in the NAB assay. The detected pre-existing low-affinity antibodies had no clinical impact.

Table 35 summarizes the results of the anti-rhG-CSF antibody assays (RIP and NAB assay).

Table 35 - Summary of antibody assay results from all ZARXIO clinical studies

Study number	Number of patients	Number of samples	Screening RIP cut-point Total binding	Number of subjects with results >cut-point in screening RIP	Number of samples with results >cut-point in confirmatory RIP	Number of positive samples in NAB assay
EP06-302	214	1583	0.64% - 0.99% [2]	2	0	N/A
EP06-109	28	81	1.81%	3	0	N/A
EP06-101	40	103	2.92%	3	0	N/A
EP06-102	26	75	0.90%	3	3 [1]	0
EP06-103	56	167	1.52%	7	0	N/A
EP06-105	24	72	0.68%	2	0	N/A
EP06-301	170	643	2.27%	14	0	N/A
Total	558	2724		34	3	

[1] All three confirmed positive samples derive from the same subject; subject was already positive at baseline, with no increase in titer after treatment.

[2] Range of run specific screening cut-points (using a validated floating cut-point).

In summary, there was no evidence for the generation of antibodies to rhG-CSF in any of the healthy volunteer or patient samples tested within the clinical program, which can be attributed to the treatment with ZARXIO, US-licensed Neupogen or EU-authorized Neupogen. This result is in line with the immunogenicity rate in clinical studies described for Neupogen and the overall low immunogenicity rate seen for endogenous G-CSF. No neutralizing antibodies after Neupogen treatment have been described and the number of binding anti-rhG-CSF antibodies is low (Amgen USA 2014). However, a direct comparison of the number of binding anti-G-CSF antibodies to results from other Neupogen clinical studies is hampered by the use of different immunogenicity assay methods with different sensitivity and accuracy. In addition, unspecific and inconclusive results for binding anti rhG-CSF antibodies have been reported from several clinical studies.

Thus the confirmed overall low immunogenicity rate of ZARXIO and US-licensed Neupogen confirmed that the immunogenicity safety profiles are similar.

8.5.3 Results from post-marketing pharmacovigilance

There is a theoretical possibility that an antibody directed against filgrastim may cross-react with endogenous G-CSF, resulting in immune-mediated neutropenia. However, no neutralizing antibodies have been reported in post-marketing experience for ZARZIO.

As described in the US prescribing information of Neupogen, in clinical studies comparing Neupogen and Neulasta the incidence of antibodies binding to Neupogen was 3% (11/333). In

these 11 patients, no evidence of a neutralizing response was observed using a cell-based bioassay (Amgen USA 2014).

Sandoz is closely monitoring the safety of the product on the market worldwide and summary reports of the post-marketing safety are generated in form of PSURs on a frequent basis.

9 Conclusions

The biosimilarity of ZARXIO to the reference product, Neupogen, has been demonstrated using multiple and overlapping *in vitro* and *in vivo* test systems. Filgrastim is a relatively simple, non-glycosylated protein, which has been produced and thoroughly characterized using state-of-the-art analytical methods. Through these methods, the structure and function of ZARXIO has been shown with a high level of confidence to be highly similar to the reference product, providing adequate confidence that there will be no clinically meaningful differences between the two products. High confidence in this similarity has been further confirmed through the results of five randomized (one pivotal and four supportive), double-blind, single and multiple dose PK/PD studies in a total of 174 healthy volunteers which confirmed PK/PD equivalence of ZARXIO and Neupogen (US-licensed and EU-authorized) and one PK sub-study in 54 breast cancer patients which provided supportive comparative bioavailability data of ZARXIO and US-licensed Neupogen.

High similarity has been further demonstrated through two safety and efficacy studies [one comparative pivotal for the US file and one non-comparative for the EU approval] and a non-comparative post-authorization safety study which demonstrated no clinically meaningful difference in ZARXIO efficacy compared to the reference product. No clinically relevant immunogenicity has been observed during controlled trials with ZARXIO, which is consistent with the filgrastim experience in general. Furthermore, post-marketing safety evaluations involving in excess of 7.5 million patient-days of experience, confirm the safety of the product. Throughout this experience, no clinically meaningful differences between ZARXIO and Neupogen have been observed.

Based on the totality of the evidence presented, ZARXIO has demonstrated its biosimilarity to Neupogen, and therefore meets the regulatory requirements to be considered biosimilar to Neupogen. The findings of no clinically meaningful differences between ZARXIO and Neupogen support the view that extrapolation to the full range of indications included in the current US-Neupogen license is appropriate. In conclusion, the approval of a biosimilar requires clear demonstration of high similarity using robust and over lapping methods. Analytical methods confirm that the products, for all intents and purposes, are the same. The numerous bioassays provide further confidence that clinical responses to ZARXIO will be equivalent to Neupogen in any indication. Therefore, while only clinically evaluated breast cancer patients, approval of ZARXIO for all indications is justified.

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Appendix 1 – PK and PD results from supportive PK/PD studies

The tables below summarize the results for the PK and PD parameters in the supportive PK/PD studies together with the respective confidence intervals for the comparison between ZARXIO and Neupogen.

Table 1 – Ratios and 90% confidence intervals for the PK parameters in the supportive PK/PD studies (PP populations)

				Geometric means			
Parameter	**Study**	**Dose (mcg/kg)**	**Route**	**ZARXIO**	**Neupogen***	**Ratio (%)**	**90% CI (%)**
$AUC_{0\text{-}36h,sd}$	EP06-102	5	i.v.	632.1	634.2	99.68	[96.94; 102.47]
$AUC_{0\text{-}24h,sd}$	EP06-105	1	s.c.	58.3	65.7	91.17	[85.95; 96.72]
	EP06-103	2.5	s.c.	119.6	136.8	87.46	[80.95; 94.48]
	EP06-103	5	s.c.	370.3	383.7	95.87	[90.31; 101.78]
	EP06-101	10	s.c.	839.7	908.1	93.13	[88.76; 97.70]
$AUC_{144\text{-}168h,ss}$	EP06-103	2.5	s.c.	42.6	49.1	86.14	[80.58; 92.08]
	EP06-103	5	s.c.	106.1	121.9	86.90	[80.90; 93.35]
	EP06-101	10	s.c.	175.3	193.1	90.78	[84.45; 97.60]
$C_{max,0\text{-}36h,sd}$	EP06-102	5	i.v.	186.4	188.7	98.82	[95.76; 101.98]
$C_{max,0\text{-}24h,sd}$	EP06-105	1	s.c.	9.1	10.6	88.50	[81.55; 96.03]
	EP06-103	2.5	s.c.	16.9	19.5	86.61	[78.63; 95.40]
	EP06-103	5	s.c.	48.2	49.9	95.91	[88.73; 103.67]
	EP06-101	10	s.c.	97.7	110.3	88.84	[82.49; 95.67]
$C_{max,144\text{-}216h,ss}$	EP06-103	2.5	s.c.	7.4	9.5	77.75	[70.81; 85.40]
	EP06-103	5	s.c.	21.8	27.2	80.05	[70.19; 91.30]
	EP06-101	10	s.c.	35.0	39.1	89.68	[81.83; 98.28]

*EU-authorized Neupogen; AUC = Area under the serum concentration-time curve between the specified time points; CI = Confidence interval; C_{max} = Maximum observed serum concentration; Ratio: E2006/Neupogen; sd = single-dose; ss = steady state (multiple-dose)

Table 2 – AUEC of absolute neutrophil count and CD34$^+$ cell counts across the supportive PK/PD studies (PP populations)

Study	Dose (mcg/kg)	Route	Parameter	Geometric means		Ratio (%)	95% CI (%)
				ZARXIO	Neupogen*		
ANC (10^9×h/L)							
EP06-105	1	s.c.	$AUEC_{0\rightarrow120h}$	740.78	725.00	102.11	[96.68; 108.09]
EP06-103	2.5	s.c.	$AUEC_{0\rightarrow216h}$	4224.0	4134.5	102.16	[99.49; 104.91]
EP06-103	5	s.c.	$AUEC_{0\rightarrow216h}$	5191.8	5176.8	100.61	[98.01; 103.29]
EP06-101	10	s.c.	$AUEC_{0\rightarrow216h}$	6474.5	6515.3	99.37	[96.30; 102.54]
EP06-102	5	i.v.	$AUEC_{0\rightarrow120h}$	944.72	950.19	99.42	[93.52; 105.70]
CD34$^+$ cell count (h×cells/µL)							
EP06-103	2.5	s.c.	$AUEC_{0\rightarrow216h}$	2815.1	2694.0	104.49	[96.51; 113.14]
EP06-103	5	s.c.	$AUEC_{0\rightarrow216h}$	2885.5	2898.3	98.99	[86.79; 112.90]
EP06-101	10	s.c.	$AUEC_{0\rightarrow216h}$	5129.3	5023.3	102.11	[94.93; 109.83]

*EU-authorized Neupogen; AUEC = Area under the effect-time curve between the specified time points, E_{max} = Maximum observed effect; Ratio = ZARXIO/Neupogen

Appendix 2 – Patient Demographics

Demographics in human studies

Healthy volunteer studies

Table 1 - Summary of baseline demographic data of the subjects in the Phase I studies

Parameter	N	Mean	SD	CV	Minimum	Median	Maximum
EP06-109							
Age (years)	28	37.1	6.9	18.6	19	38.0	49
Weight (kg)	28	72.6	9.9	13.5	55.2	70.3	93.8
BMI (kg/m^2)	28	25.2	2.5	9.8	19.6	25.9	29.7
Height (cm)	28	169.4	7.8	4.6	153.0	169.7	189.5
EP06-101							
Age (years)	40	35.2	5.6	16.0	25	36.0	45
Weight (kg)	40	69.9	9.1	13.1	55.7	68.5	89.1
BMI (kg/m^2)	40	23.3	1.8	7.7	19.2	23.2	27.0
Height (cm)	40	173.0	9.7	5.6	155	174.0	193
EP06-102							
Age (years)	26	30	5	18.3	23	28	39
Weight (kg)	26	75.9	10.6	13.9	58.5	74.5	101.0
BMI (kg/m^2)	26	24.4	1.9	7.6	21.4	23.9	27.8
Height (cm)	26	176.1	9.3	5.3	162.0	175.6	198.5
(continued on next page)							
EP06-103 Dose group 1 (2.5 µg/kg/day)							
Age (years)	28	37	10	27.8	22	33	54
Weight (kg)	28	79.2	13.1	16.6	55.5	79.5	111.8
BMI (kg/m^2)	28	24.9	2.1	8.4	20.2	25.6	27.2
Height (cm)	28	177.6	10.5	5.9	155.0	177.8	205.0
EP06-103 Dose group 2 (5 µg/kg/day)							
Age (years)	28	40	8	21.2	21	42	53
Weight (kg)	28	71.2	10.1	14.2	54.9	70.1	93.1
BMI (kg/m^2)	28	23.6	2.3	9.7	19.5	22.8	28.4
Height (cm)	28	173.5	7.1	4.1	162.0	175.0	191.0
EP06-105							
Age (years)	24	40	9	22.5	21	42	53
Weight (kg)	24	76.3	11.6	15.2	61.2	77.8	95.1
BMI (kg/m^2)	24	24.7	2.0	8.1	21.6	24.9	27.3
Height (cm)	24	175.0	9.3	5.3	158.0	176.0	190.0

Studies in breast cancer patients (EP06-302 & -301)

Table 2 - Baseline characteristics in pivotal study EP06-302 FAS/SAF

	ZARXIO (Cycle 1)	**Neupogen (Cycle 1)**	**Unswitched (All Cycles)**	**Switched (All Cycles)**	**ZARXIO (All Cycles)**	**Neupogen (All Cycles)**
	N=107	N=107	N=105	N=109	N=53	N=52
Age (years)						
Mean (SD),	49.5 (11.52)	48.4 (11.02)	49.2 (11.22)	48.6 (11.35)	51.5 (11.16)	46.9 (10.91)
Time (months) since initial diagnosis of breast cancer						
Median (min, max)	1.0 (0, 171[a])	1.0 (0, 16)	1.0 (0, 171[a])	1.0 (0, 16)	1.0 (0, 171[a])	1.0 (0,7)
Stage at initial diagnosis of breast cancer, n (%)						
I	7 (6.5)	8 (7.5)	9 (8.6)	6 (5.5)	5 (9.4)	4 (7.7)
II	57 (53.3)	53 (49.5)	49 (46.7)	61 (56.0)	24 (45.3)	25 (48.1)
III	43 (40.2)	46 (43.0)	47 (44.7)	42 (38.5)	24 (45.3)	23 (44.2)

FAS: Full analysis set
SAF Safety set
SD: Standard deviation

[a] The patient experienced relapse and was operated one month before randomization however the initial diagnosis was 171 months before randomization

Table 3 - Baseline characteristics in single-arm study EP06-301 as compared to similar studies with Neupogen

	EP 2006 5µg/kg in study EP06-301	**Filgrastim 5µg/kg in Holmes study I[a]**	**Filgrastim 5µg/kg in Holmes study II[b]**	**Filgrastim 5µg/kg in Green study[c]**
No. of patients	170	25	147 efficacy / 151 safety	75 efficacy / 76 safety
Age (years) [mean (SD)]	52 (10)	50 (9)	52 (11)	53 (12)
Race [n (%)]				
White	170 (100)	20 (80)	117 (80)	73 (97)
Black	0 (0)	2 (8)	16 (11)	-
Hispanic	0 (0)	2 (8)	7 (5)	-
Other	0 (0)	1 (4)	7 (5)	-

Disease stage [n (%)]				
Stage II	5 (3)	6 (24)	72 (49)	23 (31)
Stage III	113 (66)	9 (36)	38 (26)	20 (27)
Stage IV	51 (30)	10 (40)	37 (25)	32 (43)
missing	1 (1)	0 (0)	0 (0)	0 (0)

[a] (Holmes 2002a) [b] (Holmes 2002b) [c] (Green 2003)

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Appendix 3 – Supportive Clinical Studies

Single-arm clinical safety & efficacy study – EP06-301

Study EP06-301 was an open, single-arm, multi-center study designed to evaluate safety and efficacy of ZARXIO in breast cancer patients receiving four cycles of doxorubicin and docetaxel chemotherapy. The administered chemotherapy is associated with a high risk for severe neutropenia.

Each patient was expected to participate in the study for approximately 6 months, including three months of active treatment (4 treatment cycles) and 3 months of follow-up after the last treatment cycle. The study was conducted in 170 patient at 24 centers in Russia (17 centers) and Ukraine (7 centers).

Key inclusion criteria:

- Chemotherapy-naïve patients with documented locally advanced/advanced breast cancer or patients with high risk stage II breast cancer.
- Women ≥18 years and with estimated a life expectancy of more than 6 months and ECOG performance status ≤2

Key exclusion criteria:

- Previous treatment with any G-CSF preparation.
- Concurrent or prior radiotherapy within 4 weeks of Day 1, Cycle 1.
- Clinically significant cardiac dysfunction at the time of screening, clinically significant findings on echocardiogram (EF <50%) or a history of myocardial infarction or heart failure within 6 months preceding the first treatment cycle.
- Prior bone marrow or stem cell transplant.

On Day 1 of each chemotherapy cycle, patients received an intravenous bolus infusion of doxorubicin (60 mg/m^2) followed by a 1-hour intravenous infusion of docetaxel (75 mg/m^2 approximately 1 hour later). Treatment with ZARXIO started on Day 2 of each chemotherapy cycle for up to 14 days (or until ANC reached 10×10^9/L), repeated for up to 4 cycles. ZARXIO was administered at 300 mcg (30 MU) to women weighing <60 kg and at 480 mcg (48 MU) to women weighing ≥60 kg. Full-dose chemotherapy on Day 1 of the next cycle (Day 22-25 of the previous cycle) was not to be started unless the patient had an ANC $>1 \times 10^9$/L and a platelet count $>100 \times 10^9$/L.

Study objectives and endpoints

The study objective was to evaluate safety, tolerability and efficacy of ZARXIO as primary prophylaxis of severe neutropenia in patients with breast cancer treated with doxorubicin and docetaxel. The following endpoints were evaluated:

Efficacy:

- Duration of severe neutropenia in cycles 1 to 4. The duration of severe neutropenia was calculated as the number of consecutive days with ANC $<0.5 \times 10^9$/L for each cycle.
- Incidence of febrile neutropenia (oral temperature ≥38.2°C and ANC $<0.5 \times 10^9$/L on the same day or the day after temperature elevation).
- Time to neutrophil recovery (ANC $\geq 2.0 \times 10^9$/L) was calculated for patients who developed neutropenia (ANC $<2.0 \times 10^9$/L) for each cycle.

Safety:

- Incidence, occurrence, and severity of the most common adverse reactions associated with rhG CSF treatment

Immunogenicity:

- Anti rhG-CSF antibody formation

As this was an open-label, single-arm study, all data were summarized using descriptive statistics. Patient disposition, demographics, and baseline characteristics were summarized. Incidences of adverse events were calculated by period and by chemotherapy cycle. Two-sided 95% CIs were derived for incidences of the most common adverse events associated with rhG-CSF treatment.

With a sample size of 150 patients, the expected width of the 95% CIs for estimated incidences was estimated as follows:

- Approximately 15% points for musculoskeletal pain based on an expected incidence between 26% and 42%
- Approximately 6.5% points for the proportion of patients developing binding antibodies to rhG-CSF based on the expected 3% incidence.

The safety population defined as all patients who received at least one dose of study drug was used for statistical evaluation of the primary objectives. The intent-to-treat (ITT) population was defined as all patients who received study drug for at least three days in at least one treatment cycle and was used for statistical evaluation of secondary objectives related to efficacy.

Results

The study was conducted at 24 research centers in Russia (17 centers) and Ukraine (7 centers). The planned enrollment of 170 patients was met, and 153 (90%) patients completed four chemotherapy cycles. No patients were excluded from analysis due to protocol violations.

The study population consisted of 170 Caucasian women who ranged in age from 24 to 78 years with a mean (SD) of 52.1 (10.2) years and median of 52 years. Body mass index ranged from 18.2 to 47.2 kg/m^2 with a mean (SD) of 28.1 (5.4) kg/m^2 and a median of 28.0.

Incidence and duration of severe neutropenia

The incidence and duration of severe neutropenia are summarized in Table 1. Severe neutropenia (CTC Grade 4) was observed in 80 (47.1%), 25 (15.4%), 33 (20.8%), and 27 (17.5%) patients during cycles 1, 2, 3, and 4, respectively. Severe neutropenia was observed in 90 patients (52.9 %) across all cycles.

The median DSN, defined as number of days from the first ANC $<0.5 \times 10^9$/L to the first ANC $\geq 1.0 \times 10^9$/L, was short (2 days) in each treatment cycle.

Table 1 – Supportive study EP06-301: Incidence and duration of severe neutropenia by treatment cycle

		Duration of severe neutropenia[1]		
		Incidence	Time (days) to ANC $\geq 1.0 \times 10^9$/L[2]	Consecutive days[3]
Cycle	N	n (%)	Mean ± SD	Mean ± SD
1	170	80 (47.1%)	2.2 ± 0.9	1.8 ± 1.4
2	162	25 (15.4%)	1.8 ± 0.6	1.3 ± 0.5
3	159	33 (20.8%)	1.9 ± 0.9	1.4 ± 0.6
4	154	27 (17.5%)	2.1 ± 0.8	1.7 ± 0.6

[1] Includes only patients who experienced severe neutropenia
[2] Duration defined as the number of days from the first day with ANC $<0.5 \times 10^9$/L to the first day with ANC $\geq 1.0 \times 10^9$/L
[3] Duration was the number of consecutive days with ANC $<0.5 \times 10^9$/L during the treatment cycle

Thirteen (7.6%) patients experienced FN during the first cycle. One additional patient experienced febrile neutropenia in the third cycle. The incidence of neutropenia (ANC $<2.0 \times 10^9$/L) and the time to neutrophil recovery are summarized in Table 2.

The incidence of neutropenia was 85% during the first treatment cycle and approximately 70% in cycles 2 to 4. The mean time to neutrophil count recovery (ANC $\geq 2.0 \times 10^9$/L) in each cycle was approximately 8 days after the start of chemotherapy and approximately two days after the ANC nadir.

Table 2 – Supportive study EP06-301: Incidence of neutropenia and time to neutrophil recovery by treatment cycle

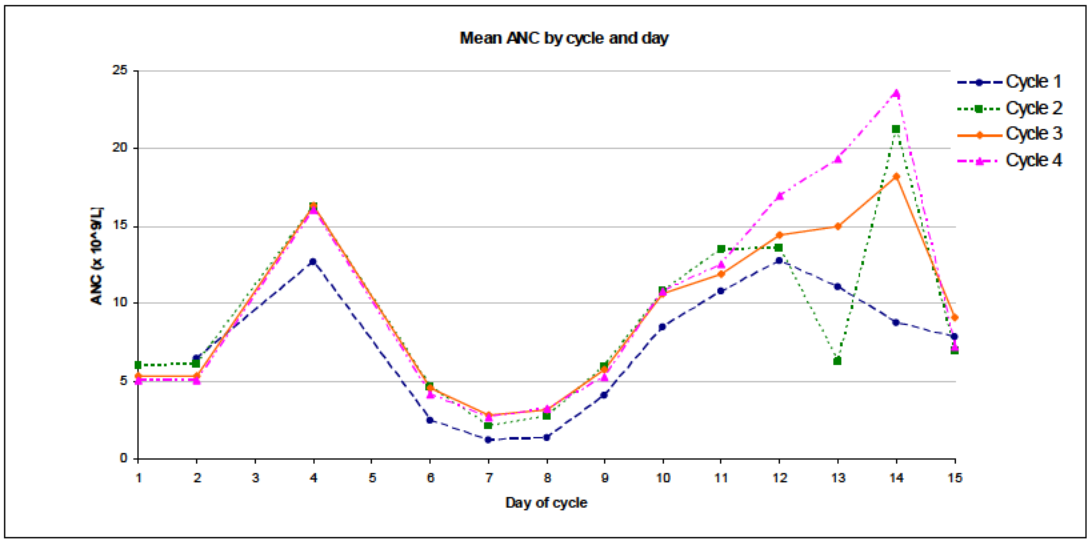
		Incidence of Neutropenia[1]		Time to Recovery (days)[2]	
		Total	Recovered	From start of chemotherapy	From ANC nadir
Cycle	N	n (%)[3]	n (%)[4]	Mean ± SD	Mean ± SD
1	170	144 (84.7%)	142 (98.6%)	8.4 ± 1.1	2.0 ± 0.8
2	162	111 (68.5%)	109 (98.2%)	7.8 ± 1.2	1.7 ± 0.8
3	159	113 (71.1%)	112 (99.1%)	8.2 ± 2.3	1.7 ± 0.8
4	154	111 (72.1%)	111 (100%)	8.0 ± 1.5	1.9 ± 1.0

[1]Neutropenia was defined as ANC $<2.0 \times 10^9$/L
[2]Recovery was defined as the first day after the nadir with ANC $\geq 2.0 \times 10^9$/L
[3]Percent is based on the number of patients treated in the cycle.
[4]Percent is based on the number of patients who developed neutropenia in the cycle.

Time course of ANC

The mean ANC curve for each cycle was congruent for all cycles from Day 1 to Day 11. The depth of the nadir was greatest in Cycle 1 compared with the subsequent three cycles and in line with the typical ANC profile under G-CSF treatment (Figure 1**Error! Reference source not found.**).

Figure 1 - Supportive study EP06-301: Mean ANC curve for each cycle



By Day 11 (Cycles 2 to 4) or Day 12 (Cycle 1) the ANC of the vast majority of patients had reached at least 10×10^9/L and therefore the treatment with ZARXIO was stopped in these patients. Thus, due to the limited number of ANC measurements at these time points the ANC

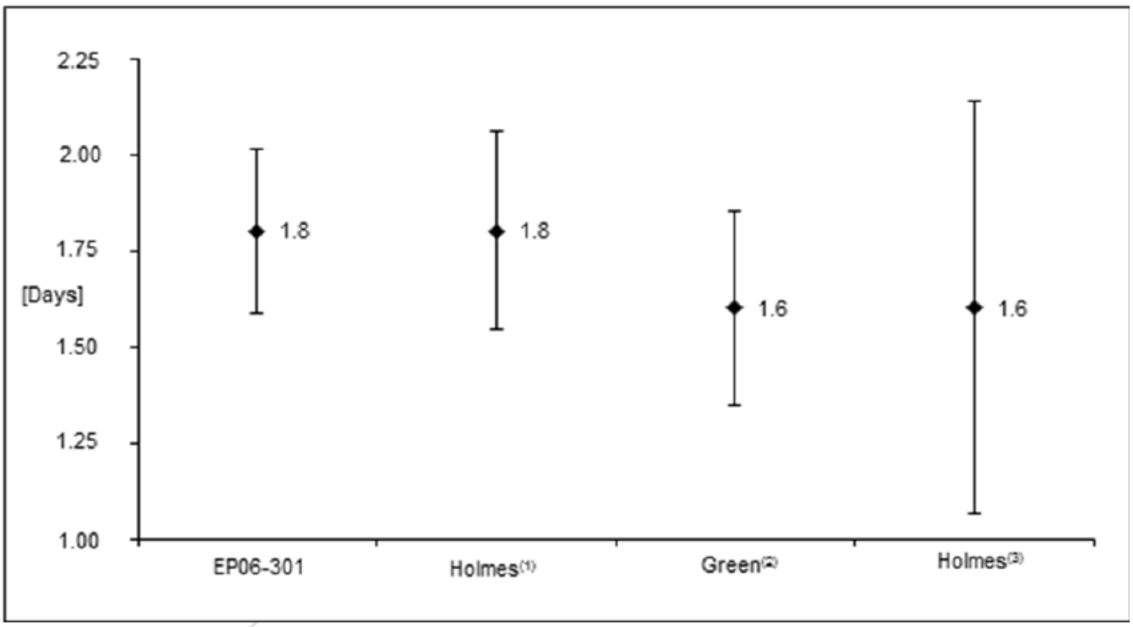
curves from Day 12 to Day 15 are more variable impairing a proper comparison of the cycles from Day 12 onwards.

Comparison of the EP06-301 efficacy results with literature data of Neupogen

The non-comparative effectiveness results of study EP06-301 were put into perspective using published results of Neupogen (Figure 1), summarizing the results of three studies with Neupogen treatment arms in breast cancer patients receiving the same chemotherapy regimen (doxorubicin 60 mg/m^2 and docetaxel 75 mg/m^2) (Holmes 2002a, Green 2003, Holmes 2002b).

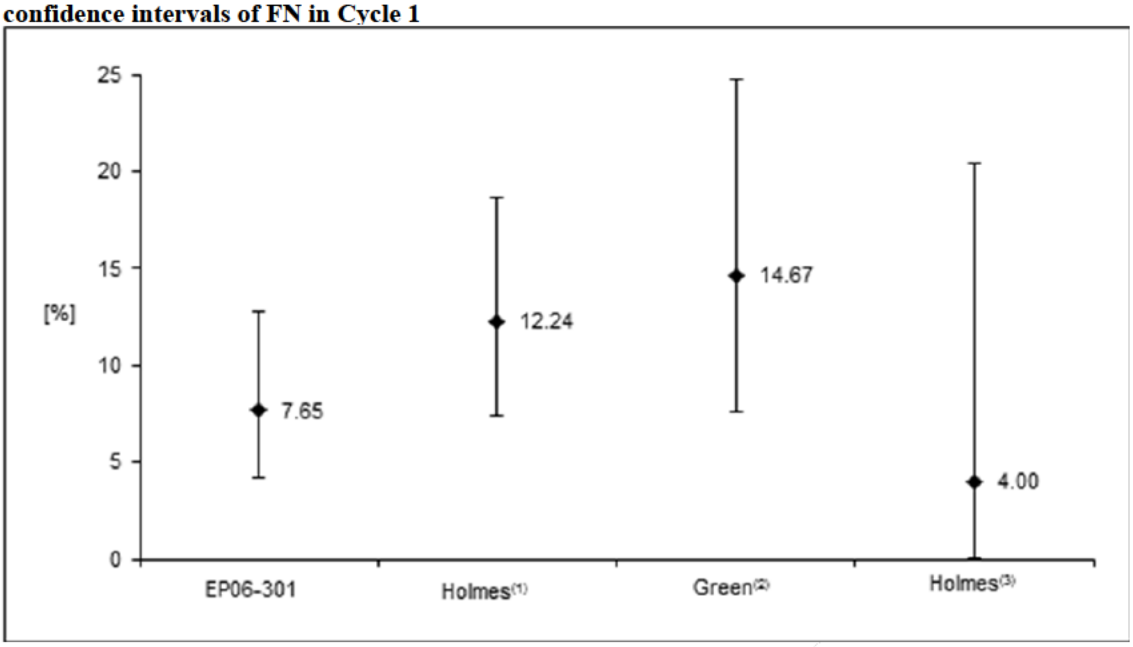
A similar duration of severe neutropenia in Cycle 1 between these Amgen studies and the results of study EP06-301 can be noted in Figure 2. The mean duration of severe neutropenia was 1.8 days in the ZARXIO treated patients matching the available information for Neupogen treated patients. Figure 3 depicts the reported incidences of febrile neutropenia and results from the EP06-301 study.

Figure 2 - Comparison of study EP06-301 to published data for Neupogen: DSN in Cycle 1 - means and 95% confidence intervals



(1) Holmes 2002a (2) Green 2003 (3) Holmes 2002b

Figure 3 - Comparison of study EP06-301 to published data for Neupogen: Incidence rates and exact 95% confidence intervals of FN in Cycle 1



(1) Holmes 2002a (2) Green 2003 (3) Holmes 2002b

In summary, also study EP06-301 showed that the effectiveness of ZARXIO is comparable with published results for Neupogen.

Post-authorization study EP06-501 in healthy donors

Study design

Study EP06-501 has been designed in accordance with the EU post-approval risk management plan of EP2006 following a request of the European Medicines Agency. It is an ongoing non-interventional, open-label, non-controlled PASS that collects data in a target of 200 healthy unrelated stem cell donors within routine clinical practice.

This PASS provides long-term safety data on ZARXIO in healthy adult unrelated stem cell donors undergoing PBPC mobilization prior to allogeneic transplantation (approved indication for filgrastim in the EU). In addition, the effectiveness of the mobilization with ZARXIO in healthy donors is investigated. Enrolled donors are monitored for safety and effectiveness during the mobilization period, and a systematic safety data follow-up is implemented for up to 10 years after mobilization.

The primary objective of this PASS is to investigate the safety profile of ZARXIO in healthy unrelated donors. The secondary objective is to assess the effectiveness of PBPC mobilization with ZARXIO in terms of the $CD34^+$ cell count.

All healthy donors are mobilized according to the EU label of EP2006 (Zarzio SmPC 2013). The donors receive s.c. injections of ZARXIO with a dose not exceeding 10 mcg/kg/day during the

mobilization period. The apheresis should be started at day 5 and continued until day 6 if needed in order to collect 4×10^6 $CD34^+$ cells/kg recipient body weight.

Results

In the following, interim results of the study are presented. By the cut-off date 28-Aug-2013, 121 donors were enrolled, of whom 89 (73.6%) were men and 32 (26.4%) were women. The age span was 19 to 58 years; the mean age was 36.84 ± 9.31 years. ZARXIO was utilized as recommended by the EU label.

The effectiveness results in terms of $CD34^+$ harvesting in peripheral blood are summarized in Table 3.

Table 3 - Study EP06-501: Mobilization of stem cells and CD34+ cell yield after apheresis (interim data: May 2011 to August 2013)

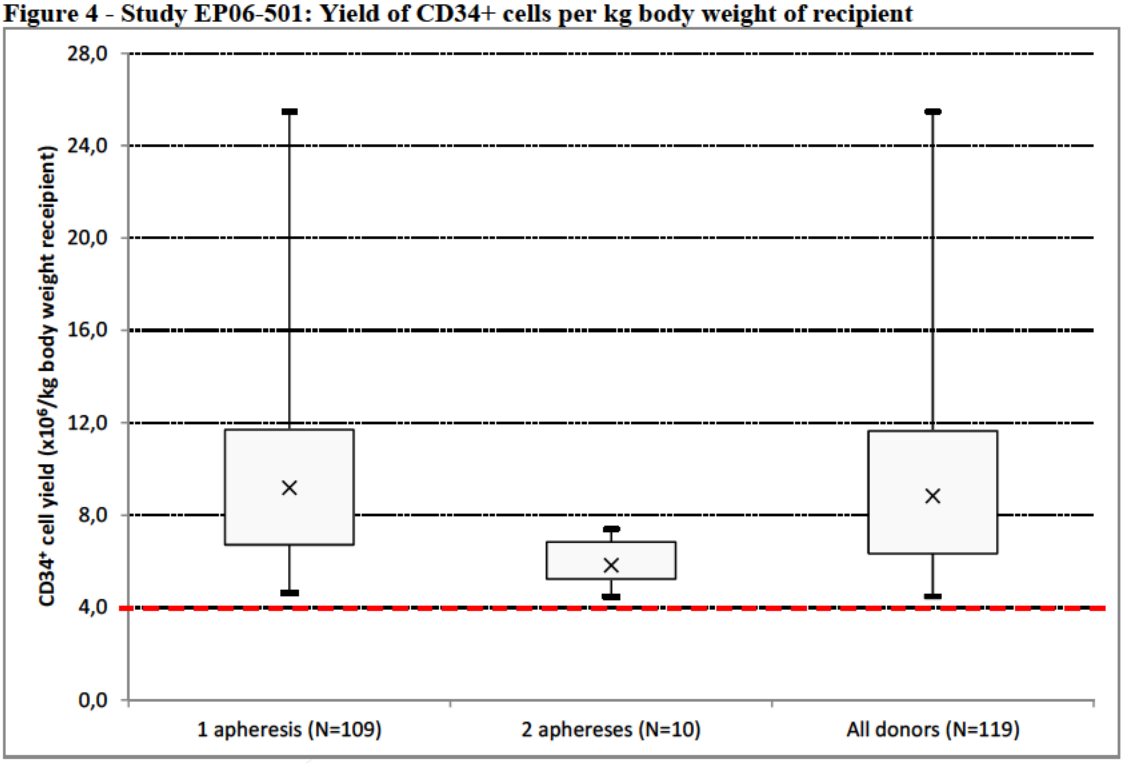
	Donors with 1 apheresis N=109	**Donors with 2 apheresis N=10**	**All donors with apheresis N=119**
$CD34^+$ cell count before apheresis (/µL peripheral blood)			
Mean ± SD	114.14 ± 55.43	57.00 ± 17.49	109.35 ± 55.58
Median (range)	106.0 (34-284)	52.5 (36-84)	100 (34-284)
$CD34^+$ cell yield after 1st apheresis ($x10^6$)			
Mean ± SD	718.20 ± 290.32	353.20 ± 71.84	687.53 ± 296.44
Median (range)	683.0 (274-1656)	359.5 (204-453)	663.0 (204-1656)
$CD34^+$ cell yield after 2nd apheresis ($x10^6$)			
Mean ± SD	not applicable	203.20 ± 94.33	not applicable
Median (range)		200.0 (80-358)	
$CD34^+$ cell yield in total ($x10^6$)			
Mean ± SD	718.20 ± 290.32	556.40 ± 148.78	704.60 ± 284.37
Median (range)	683.0 (274-1656)	551.5 (295-765)	673.0 (274-1656)
$CD34^+$ cell yield per kg recipient in total ($x10^6$/kg)			
Mean ± SD	9.69 ± 3.77	5.96 ± 0.94	9.37 ± 3.76
Median (range)	9.19 (4.64-25.48)	5.84 (4.48-7.40)	8.84 (4.48-25.48)

Note: N=119 donors were evaluable: apheresis was cancelled in one donor, and some data are currently missing for another donor. Interim data May 2011 to Aug 2013 (cut-off date 28-Aug-2013)

Generally, the extent of the PBPC mobilization prior to apheresis was highly variable, with an inter-individual range of 34 to 284 $CD34^+$ cells/µL peripheral blood. As expected the $CD34^+$ cell count in the peripheral blood prior to first apheresis was clearly lower among the donors undergoing two apheresis sessions compared to those with one apheresis session.

Table 3 and Figure 4 show that that PBPC mobilization with ZARXIO was successful in the evaluable 119 donors as in all these subjects the $CD34^+$ yield was above the threshold of 4×10^6 cells/kg body weight recipient as defined in the EU label (Zarzio SmPC 2013). This goal was met with one single apheresis session in about 90% of the donors. The data generated in this study confirm the effectiveness of ZARZIO in the mobilization setting as already suggested by the results seen for the $CD34^+$ cell response seen in the healthy volunteer PK/PD studies.

Figure 4 - Study EP06-501: Yield of CD34+ cells per kg body weight of recipient



Provided are, per number of apheresis and in the total population, the minimum and maximum values (lower and upper whisker, respectively), 25%- and 75%-quartiles (lower and upper horizontal bar margin, respectively), and median (cross within bars). The horizontal dashed line visualizes the desired minimum yield of at least 4×10^6 cells/kg body weight recipient. Interim data May 2011 to Aug 2013 (cut-off date 28-Aug-2013)

Appendix 4 – Zarzio – Supportive European Experience

Several phase IV studies have evaluated the use of Zarzio for the prevention of chemotherapy-induced neutropenia or hematopoietic stem cell mobilization in clinical practice in Europe. These studies were not included in the US BLA. Safety and effectiveness results from these trials are being provided so that Sandoz is completely transparent to the Advisory Committee with the data available to the company.

Results from these studies are consistent with the filgrastim safety and effectiveness profile observed in studies included in the BLA as well as with the filgrastim safety and effectiveness profile reported in the literature for the reference product.

Chemotherapy-induced neutropenia

Two large prospective multicenter studies assessed the use of Zarzio for the prevention of neutropenia in patients with cancer receiving chemotherapy. The MONITOR G-CSF study was a non-interventional study that evaluated 1447 patients who received Zarzio as neutropenia prophylaxis for up to 6 cycles within a single chemotherapy line (n= 6213 cycles).[1] The most frequent tumor types in patients included breast cancer (32%), lung cancer (24%) and lymphoma (17%). Chemotherapy-induced neutropenia occurred in 14.3% of all cycles and 34.8% of patients had ≥1 episode. Twenty-three percent of patients had ≥1 episode of severe (grade 3 or 4) neutropenia and 5.9% experienced febrile neutropenia. Neutropenia-related hospitalizations occurred in 6.1% of patients and disruptions to chemotherapy (i.e. dose reduction, delay or cancellation) occurred in 9.5% of patients. In the safety sample (1496 patients with 6392 cycles) there were 148 ADRs reported (2.3% of cycles) in 76 (5.1% of) patients (Sandoz data on file). The majority of ADRs were mild or moderate (124/148; 83.8%) and resolved completely (142/148; 95.9%). Of the 148 ADRs, the most frequent were bone pain (23.0%), arthralgia (14.2%), myalgia (7.4%), diarrhea (6.8%), back pain (4.7%) and rash (4.7%) (Table 1). Four serious ADRs (4/148 ADRs or 2.7%; 4/1496 patients or 0.3%) were reported, these being bone pain, drug hypersensitivity, vulval abscess and loss of consciousness. There were no neutropenia-related deaths and no Zarzio-related deaths.

The HEXAFIL study was also a prospective non-interventional study that assessed the safety and efficacy of Zarzio in routine clinical practice in Germany.[2] An interim analysis of the first chemotherapy cycle included 955 patients (of a final valid case cohort of 1337), with the most frequent tumor types being breast cancer (57.2%), non-Hodgkin's lymphoma (10.7%), lung cancer (7.6%), ovarian cancer (4.0%) and Hodgkin's lymphoma (3.1%). Only 1.9% of all patients experienced febrile neutropenia, while 8.7% had neutropenic complications and 14% had leukopenia CTC 4 at nadir. Overall, 6% of patients had chemotherapy disruptions (modified in 4.6% and discontinued in 1.4% of patients). Safety data were reported for a safety cohort of 1469 patients.

A total of 119 patients (8.1%) experienced at least one ADR (German Study Register DRKS: www.drks.de; study ID: DRKS00000313; accessed 3 November, 2014). Overall, 163 ADRs were reported in total. The most frequent was pain (affecting 6.9% of patients), with the vast majority of cases being back or bone pain (5.0%) (Table 2). The majority of ADRs were of a mild to moderate grade with 106 patients (7.2%) experiencing CTC grade 1 or 2 ADRs and 11 (0.7%) experiencing a grade 3 or 4 event. One patient had a fatal serious AE (SAE) of myocardial infarction and hypoxic brain injury. This SAE occurred in cycle 3 and was not considered to be

related to filgrastim treatment. Two serious ADRs were reported, one of gastrointestinal pain (grade 2) and one of bone pain (grade 3). Both of these SAEs occurred in cycle 1 and were considered to be related to filgrastim treatment. Both patients made a complete recovery (Sandoz data on file).

Three single-center non-interventional studies have also reported the use of Zarzio for prevention of chemotherapy-induced neutropenia in patients with solid tumors and hematological malignancies.[3-5] These studies, including conclusions drawn with respect to product safety and effectiveness, are summarized in Table 3.

Hematopoietic stem cell mobilization

A considerable number of single-center, non-interventional studies have assessed the use of Zarzio for hematopoietic stem cell mobilization. These studies have primarily included patients undergoing mobilization for autologous transplant in patients with lymphoma, multiple myeloma and other hematological malignancies (Table 4); however, a few studies have reported data for mobilization in healthy individuals for allogeneic transplant (Table 5).

Safety and effectiveness conclusions from these studies are incorporated into the tabular summaries of Table 4 and Table 5

Table 1 - ADRs (>3%) in the MONITOR G-CSF study

	ADRs, n (% of 148 ADR)
Bone pain	34 (23.0)
Arthralgia	21 (14.2)
Myalgia	11 (7.4)
Diarrhea	10 (6.8)
Back pain	7 (4.7)
Rash	7 (4.7)

Table 2 - ADRs (experienced by ≥3 patients) in the HEXAFIL study (n=1469)

	ADRs, n (%)
Pain	101 (6.9)
Musculoskeletal: back or bone pain	74 (5.0)
Other pain	6 (0.4)
Musculoskeletal limb pain	5 (0.3)
Neurologic pain / Headache	4 (0.3)
Gastrointestinal pain	3 (0.2)
Pain	2 (0.1)
Musculoskeletal joint pain	2 (0.1)
Musculoskeletal muscle pain	2 (0.1)
Cardiovascular pain / Pericardium	1 (0.1)
Musculoskeletal neck pain	1 (0.1)
Musculoskeletal / soft tissue	11 (0.7)
Constitutional symptoms	11 (0.7)
Dermatology / skin	5 (0.3)
Gastrointestinal	4 (0.3)
Neurology	3 (0.2)
Pulmonary / upper respiratory	3 (0.2)

Table 3 - Single-center non-interventional studies of Zarzio for the prevention of chemotherapy-induced neutropenia

Study	Patients (n)	Cancer types	Efficacy	Safety
Verpoort et al, 2012[3]	77	Breast, n=22 Lymphoma/leukaemia, n=17 Colon, n=10 Other, n=28	One patient developed febrile neutropenia. Neutropenia led to chemotherapy dose reductions in 5 patients (6.5%) and discontinuation in 2 patients (2.5%). Results were comparable to a historical control cohort (n=25) in which one patient developed febrile neutropenia and neutropenia led to chemotherapy dose reductions in 2 patients (8%) and dose discontinuation in 2 patients (8%).	No unexpected safety findings were observed.
Salesi et al, 2012[4]	48	Lung, n=17 Colorectal, n=11 Breast, n-10 Other solid, n=10	Febrile neutropenia was observed in 3 patients. Six patients had non-febrile grade 4 neutropenia.	No unexpected adverse effects were reported.
Rosati et al, 2011[5]	42	Breast, n=15 Other solid, n=27	Severe neutropenia was recorded in 24/401 blood tests. No patients developed febrile neutropenia.	Musculoskeletal events (arthralgia, myalgia, bone pain) were as expected.

Table 4 - Studies of Zarzio in patients undergoing autologous hematopoietic stem cell mobilization (including neutrophil recovery after HSCT)

Study design and no. of patients	Mean/median duration of G-CSF (days)	Mean/median number of leukapheresis*	Mean/median no. of CD34+ cells mobilized by body weight ($\times 10^6$/kg)	Mean/median no. of CD34+ cells mobilized in PB ($\times 10^6$/μL)†	Safety /AEs
Comparative study of Zarzio (n=40) vs historical originator (n=41)[6]	Zarzio: 5 (5–12) Originator: 5 (5–9)	Zarzio: 1 (1–3) Originator: 1 (1–3)	Zarzio: 5.50 (1.1–20) Originator: 4.49 (0.9–25)	Zarzio: 55.5(1–196) Originator: 60.0 (13–432)	*Bone pain and/or headache:* Zarzio 5 μg: n = 3 Zarzio 10 μg: n = 11 Originator: N/A
Comparative study of Zarzio (n=54) vs originator (n =54)[7]	Zarzio: 8 (4–17) Originator: 8 (4–14)	Zarzio: 1 Originator: 1	Zarzio: 9.1 (0–23) Originator: 9.4 (6–48)	Zarzio: 62.0 (2–394)Originator: 47.5 (2-370)	*Bone pain:* Zarzio: n = 8 Originator: n = 6
Comparative single-center study of Zarzio (n=26) vs historical originator (n=48)[8]	Zarzio: 16.5 (11–44) Originator: 15.0 (9–23) (until neutrophil engraftment; >500/μL)	N/A	Zarzio: 9.7 Originator: 8.0	Zarzio: 92/μL (Day 5) Originator: 88/μL (Day 5)	The occurrence and intensity of bone pain was similar in both groups
Non-comparative study of Zarzio (n=44)[9]	Zarzio: 8.2	Zarzio: 1.45	Zarzio: 4.3 (0.8–6.2)	Zarzio: 58.3 (10–503.5)	N/A
Comparative study of Zarzio (n=104) vs historical lenograstim (n=155)[10]	Zarzio: 13–14 Lenograstim: 12–13 (until ANC recovery: >0.5×10^9/L)	Zarzio: 1–2 (1–3) § Lenograstim: 1–2 (1–3)	Zarzio: 3.9–8.7 (0.5–29.9)§ Lenograstim: 3.1–5.1 (1.1–33.7)§	Zarzio: 25.9–66.7 (6.6–577.7) § Lenograstim: 20.5–32.5 (5.1–412.9) §	N/A
Comparative study of Zarzio (n=10) vs historical originator (n=10)[11]	Zarzio: 12 (10–16) Originator: 14 (10–17) (time to leucocyte engraftment)	Zarzio: 1 (1–3) Originator: 1 (1–2)	Zarzio: 4.10 (0.25–4.84) Originator: 2.71 (1.22–10.3)	N/A	*Bone pain:* Zarzio: n = 3 Originator: n = 2
Comparative study of Zarzio (n=36) vs historical originator (n=36)[12]	Zarzio: 5 (5–6) Originator: 5 (5–6)	Zarzio: 1 (1–3) Originator: 1 (1–3)	Zarzio: 4.76 (2.04–13.42) Originator: 4.36 (1.04–10.98)	Zarzio: 58.8 (3.2–256.5) Originator: 31.3 (1.8–119.1)	Mild bone/muscle pain in both groups
Non-comparative study of Zarzio (n=40)[13]	Zarzio: 12 (9-27)	N/A	Zarzio: 5.2 (2.22-57.07)	N/A	
Comparative study of Zarzio (n=80) vs historical lenograstim (n=61)[14]	Zarzio: 6 (4–13) Lenograstim: 6 (4–14)	N/A	Zarzio: 3.6 (0-47) Lenograstim 3.4 (0.1-45)		Adverse events included bone pain, headache and/or neutropenic fever: Zarzio: n=17

					Lenograstim: n=15
Neutrophil recovery after autologous HSCT					
Comparative study of Zarzio (n=55) vs historical originator (n=35)[15]	Until ANC recovery: >0.5×10^9/L: Zarzio: 12 (10−13) Originator: 12 (10−21)(	Zarzio: N/A Originator: N/A	Zarzio: 6.7 ± 3 Originator: 3.9 ± 3	Zarzio: N/A Originator: N/A	*Infections (grade 3 or 4):* Zarzio: n = 9 Originator n = 11 *Neutropenic fever:* Zarzio n = 3 Originator, n = 7
Comparative study of Zarzio (n=23) vs historical originator[16]	Zarzio: 14.4 ± 5.1 Originator 18.6 ± 11.5	N/A	N/A	N/A	*Bone pain:* Zarzio: n = 7 Originator: n = 7 *Neutropenic fever:* Zarzio n = 7 Originator, n = 7
Non-comparative study of Zarzio (n=70)[13]	Median time to ANC engraftment: 9 (8-11) days. Median time to platelet engraftment 10.5 (7-19) day).				
Comparative study of Zarzio (n=64) vs historical originator (n=79), lenograstim (n=99) and pegfilgrastim (n=60)[17]	Zarzio: 9 (7–17) Originator: 10 (5–24) Lenograstim: 9 (4–26) Pegfilgrastim: 1	N/A	N/A	N/A	No serious AEs in any group
ANC = absolute neutrophil count; NA = data not available; PB = peripheral blood; *Ranges not provided; ‡For lymphoma and myeloma patients, respectively; §Depending on indication and age: lymphoma or myeloma patients aged < and ≥60 years.					

Table 5 - Studies of Zarzio in patients undergoing allogeneic hematopoietic stem cell mobilization

Study design and no. of donors	Mean/median duration of G-CSF (days)	Mean/median number of leukapheresis	Mean/median no. of CD34+ cells mobilized by body weight ($\times 10^6$/kg)	Mean/median no. of CD34+ cells mobilized in PB ($\times 10^6$/μL)	Safety/AEs
Comparative study of Zarzio (n=9) vs historical originator (n=9)[18]	Zarzio: 5 (5−6)	Zarzio: 1 (1−2)	Zarzio: 7.2 (4−9.2) Originator: 9.0 (6−14.3)	Zarzio: 70.2 (24−114) $\times 10^9$/L Originator: 86.3 (42.3−146.4)$\times 10^9$/L	Mild bone/muscle pain in all patients in both groups
Non-comparative study of Zarzio (n=21)[19]	Zarzio: 5 (4−7)	Zarzio: 1.5 (1−3)	Zarzio: 6.0 (2.6−9.2)	Zarzio: 72 (16−145)	Bone pain: n = 8
Non- comparative study of Zarzio (n=48)[10]	Zarzio: 16 (10−28) (until ANC recovery: $>0.5\times 10^9$/L)	Zarzio: 1−3	Zarzio: 6.1	N/A	N/A
Comparative study of Zarzio (n=26) vs historical originator (n=48)[20]	Zarzio: 5 Originator: 5	NA	Zarzio: 9.7 Originator: 8.0	Zarzio: 92 Originator: 88	N/A
ANC = absolute neutrophil count; NA = data not available					

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